INTRODUCTION

Although there is substantial evidence that acute and chronic stress are associated with changes in immune function (1–5), relationships among stress, immune changes, and disease outcomes are less clear (6–9). In addition, there are reported associations between pain and measures of immune function (10, 11), but relationships among pain, immune function, and vulnerability to disease are not well established. Investigation of these relationships is particularly relevant for patients who must undergo painful or stressful medical or dental procedures and who may be vulnerable to complications or negative health outcomes. There is also evidence that stress and vulnerability to illness may be linked (12). We previously found that patients who reported moderate to severe levels of stress during the week before RCT reported significantly more flu and cold symptoms in the subsequent month (13). The results of these studies suggest a prospective relationship between stress and disease outcome.

Several attempts have been made to establish immune and neuroendocrine mediators linking stress and disease outcomes. One of the most extensively examined immune measures associated with stress and illness is the cytotoxic function of natural killer cells (NKCC) (14–17). NK cells play an important role in viral surveillance and in the overall defense against viral infections (18, 19). It has been proposed that even limited transitory changes in the immunologic equilibrium in which NK activity fluctuates in response to psychological stressors may have negative consequences for health (20). Most studies have observed downregulation of NK activity in patients under chronic stress, but NK activity seems to be temporarily enhanced during acute stress (21). It is also possible that after an extended acute stressor there is a recovery period during which NK activity drops below some critical level, and this time point may provide a window for disease susceptibility (22).

Acute pain usually results in a suppression of NKCC (11, 23), although one study found results to the contrary. Greisen et al. (24) reported a slight increase in NKCC in response to painful electric stimulation. The increase in NKCC was blocked when local anesthetic was applied before painful stimulation. Unmanaged surgical pain also leads to suppression of NK cell activity, which seems to mediate the surgery-induced enhancement of metastatic colonization of tumor cells in rats (25). Whether and to what extent such acute

Pain and Immunologic Response to Root Canal Treatment and Subsequent Health Outcomes

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Objective: This study examined the effects of pain and stress associated with a dental procedure, root canal treatment (RCT), on natural killer cell cytotoxicity (NKCC) and the subsequent development of symptoms of upper respiratory illness during the following month. Methods: Patients (N = 33) were recruited from those scheduled for RCT appointments. Subjects for a non-RCT comparison group (N = 14) were also recruited from dental clinic patients. Peripheral blood was drawn by use of an indwelling catheter three times: just before RCT, 30 minutes after injection of a local anesthetic, and 30 minutes after RCT (a parallel time course was followed for the comparison group.) Blood was assayed for cortisol and NKCC. Subjects completed a health diary in the month after RCT.

Results: Patients showed a significant increase in NKCC between baseline and RCT and a significant decrease from RCT to after RCT, whereas the comparison group did not. The NKCC following the RCT was negatively correlated with the pain level during RCT (r = −0.48, p < .01) and pain levels 2 and 6 hours after RCT (r = −0.43, p < .05; r = −0.44 p < .05, respectively). The patient group reported significantly more illness episodes 2 weeks after RCT than the comparison group (Wilcoxon rank sum = 4.78, p = .03). Discriminant function analysis correctly classified 88% of the subjects into the illness category using predictor variables of post-RCT NKCC, stress, and pain levels during RCT (F(3, 21) = 8.23, p < .001). Conclusions: Transitory changes in NKCC associated with pain and stress may be implicated in the development of infectious disease episodes after an acute stressful event. Key words: pain, stress, natural killer cell cytotoxicity, health outcomes, dental procedures.
pain-related changes in NK cell activity are of clinical importance in humans remain to be established. Some recent data, however, suggest that short-term immunomodulation related to pain and stress may be an important predictor of short-term health-related events such as vulnerability to infectious diseases (26).

Taken together these findings suggest that there are important relationships between pain- and stress-related immune mechanisms and health outcomes. However, few studies have demonstrated immune changes and negative health outcomes in a prospective, experimentally controlled study. Because of this gap in the literature, we prospectively investigated the potential relationship among pain, immune changes, and the development of symptoms of infectious disease in a clinic setting.

This study used RCT as an experimental model to examine the mechanisms that link pain, stress, changes in immune function, and disease outcomes. Worldwide, RCT is a well-accepted and beneficial treatment for diseases of the dental pulp and the sequelae of the surrounding tissues (27). In the United States, it is estimated that the majority of adults undergo one or more RCTs during their lifetime (28). Although not usually life-threatening, the underlying disease state leading to RCT and the treatment itself involve inflammation, nociception, tissue damage, and removal of tissue common to many health problems. Because the RCT model was used to study a real-life stressor performed in relatively healthy people, it allowed for the investigation of mechanisms that in more compromised patients (such as those with HIV infection or cancer) might be masked by the disease process or its treatment. Hence, the observed changes in systemic immune markers in the RCT model are more likely to be associated with the pain and stress of treatment than the underlying disease state.

The disease state that ultimately requires RCT does not develop in a predictable pattern, and it may seem to have a sudden onset. This unpredictability can lead to intense stress among some patients. It is also common for patients to perceive of RCT as painful (29) and not to habituate despite multiple exposures (30). The RCT model offers several advantages, including experimental control in which biological compromise can be observed.

The objective of this study was to examine the effects of pain and stress associated with a dental procedure (RCT) on NKCC and the subsequent development of symptoms of upper respiratory illness during the following 4 weeks. We hypothesized that among patients, the acute stress of RCT would be associated with a rise in NKCC during the procedure and with a drop below baseline in the recovery period after the stressor. We expected to see no change among the comparison group. Furthermore, we expected to see a significant negative relationship between pain and NKCC during and after the RCT and that lower NKCC would be related to the development of infectious disease symptoms in the following month.

METHODS

Procedure

Subjects were recruited by phone from a list of patients scheduled for RCT appointments. Patients were included if they were between 21 and 70 years of age. Patients were excluded if they reported that they had active cancer, were taking oral steroids, were currently taking any type of chemotherapy agent or undergoing radiation treatment, or had undergone chemotherapy or radiation in the last 12 months. Patients who met the criteria were given detailed information about the study. To control for diurnal variation of cortisol and NKCC, only patients whose appointment was scheduled between 8:00 and 9:00 AM were included in this study (31). Subjects for the comparison group were recruited by phone from dental clinic patients previously seen in a separate dental research project but who were not currently scheduled for dental treatment. The same exclusion criteria were applied to the comparison group. All assessments were completed by 10:30 AM. Approximately 23% of the scheduled patients either did not meet the inclusion criteria or declined to participate because of time constraints.

Subjects were asked to report 75 minutes before their appointment. On arrival at the Dental Clinical Research Center, patients were seated comfortably and then completed a research questionnaire packet. Next, an IV catheter was inserted into the antecubital vein of the nondominant arm. Participants viewed a 22-minute neutral travelog video of the local area. The purpose of the video was to produce a homogenous environment and waiting period during which blood hormones could stabilize after venipuncture. The first blood draw occurred 30 minutes after placement of the catheter. To ensure the homogeneity of experimental conditions and to reduce movement artifacts, all subjects were moved by wheelchair to the treatment area. Comparison subjects came back (by wheelchair) to the center for clinical studies and viewed a second neutral 60-minute video. The second blood draw occurred 30 minutes after injection of local anesthetic for patients receiving RCT and at an equivalent time point for comparison subjects. After the RCT or 60-minute video, the patients and comparison subjects were again moved by wheelchair to the clinical research center. All subjects viewed a 30-minute video about gardens around the world, again to produce a homogenous environment before the blood draw. The third and final blood draw occurred at the end of this video, after which the IV catheter was removed. Subjects completed the research instruments; patients completed the pain questionnaire, and all subjects answered written questions about the videos they had watched. The project nurse explained the health review diary to all subjects, and for patients the pain chart was explained. The call schedule for the 2-week phone call to assess compliance with the health review diary was established. The diaries were collected 1 month later. Both patients and comparison subjects were compensated $150 for completing the study.

Psychological Measures

Iowa Dental Control Scale. A modified version of the Iowa Dental Control Index was administered as a control variable during the
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phone interview. Prior research has established this measure as a reliable predictor of the level of distress individuals experience during dental treatment (32–35).

Perceived Stress Scale. The Perceived Stress Scale is a 14-item survey used to assess the extent to which an individual feels in control vs. overwhelmed with current life stressors (36, 37). In this study, the time referent was within the last month. The scale was administered before placement of the IV catheter as a control variable.

Pain and stress assessment. Baseline pain and stress measures using a visual analog scale format were collected from the patients. Two additional measurements of patients’ pain and stress levels were collected. The first assessed the intensity of the pain, the unpleasantness of the pain, and the level of stress experienced during the dental appointment. This questionnaire was completed immediately after RCT. The second questionnaire assessed pain levels 2 hours and 6 hours after RCT and was completed at home. Both questionnaires used a visual analog format (38). Patients were instructed to mark the scale, which was anchored with “no pain at all” and “worst pain (or unpleasantness or stress) imaginable,” at each of six specific time points.

Health Review

A standardized rating instrument was used to assess the presence of specific illness symptoms and illness episodes (39). The validity of this scale was previously shown through direct comparison with physicians’ ratings of patients’ symptoms (40). Using published methodology, symptoms were weighted with a score of 1 to 3, based on the probability that they were indicative of an infectious illness. Summary scores for each week were computed by adding the assigned value for each symptom present. Episodes reaching a score of 3 or more were counted as an infectious illness episode. Thus, for each week, an individual either had an illness episode or did not, and a score of 1 or 0 was accordingly assigned. Across the 4 weeks of diary completion the greatest number of episodes possible was 4 and the least was 0. A total number of symptom scores across the 4 weeks using the weighted value were also computed. The project nurse called each subject 2 weeks after RCT to ensure compliance with charting symptoms. At the 1-month follow-up appointment, the health review was collected.

Manipulation Check

All patients and subjects were asked to answer questions about the their level of attention to the videos.

Blood Preparation and Assays

Three blood draws were prepared as follows: For NK assays, 30 ml was drawn at each time point into heparinized Vacutainer tubes (Becton-Dickinson, Rutherford, NJ). Samples were kept at room temperature and assayed immediately after completion of the RCT or at an equivalent time for comparison subjects. For the cortisol assay, 5 ml of blood was collected at each time point in Vacutainer tubes treated with ethylenediaminetetraacetic acid (Becton-Dickinson). These blood samples were immediately spun down, separated, and frozen at −70°C.

Cytolytic Activity of NK Cells

Thirty milliliters of venous blood was collected in heparinized Vacutainer tubes. Lymphocytes were isolated by centrifugation on a Histopaque gradient (specific gravity, 1.077). The peripheral blood lymphocytes were tested for cytolytic activity against the NK-sensitive K562 cells and the NK-resistant, lymphokine-activated killer cell–sensitive ZKBL target cells in a standard 4-hour 51Cr-release assay as reported previously (41). Effector peripheral blood lymphocytes were dispensed into wells of 96-well microtiter plates in two-fold dilutions. Target cells labeled with Na235CrO4 were added to each well. The cell mixtures were incubated for 4 hours and then centrifuged at 500 rpm. Subsequently, 150 µl of the supernatant fluid was removed and counted in a Beckman gamma counter. Lysis of K562, but not ZKBL, was indicative of NK activity of the lymphocytes. The calculation of lytic units was used as the final measure of NK activity (42, 43).

Cortisol

Cortisol is commonly measured to assess activation of the neuroendocrine response. Cortisol, the primary glucocorticoid produced by the adrenal cortex, was assayed by radioimmunoassay (Double Antibody Cortisol RIA, Diagnostic Products Corporation, Los Angeles, CA). The assay procedure is a standard competitive radioimmunoassay in which 125I-labeled cortisol competes with cortisol in the sample for a limited number of binding sites with the antibody. Patient sample concentrations were read from a calibration curve. Approximate sensitivity of the assay is 0.3 µg/dl. Inter-assay coefficients of variation range from 5.8% to 7.8%, and intra-assay coefficients of variation range from 2.4% to 4.5% (44). Expected values for blood samples collected in the morning are 5 to 25 µg/dl (44).

Data Analysis

All distributions were tested for normality, and logarithmic transformations were applied when necessary. Raw (nontransformed) values are presented in Figure 1 for illustration purposes, but analyses of NKCC and cortisol were conducted on transformed scores. Illness symptoms and episodes were analyzed using the Wilcoxon rank sum test.

To assess whether there were changes over time, differences between groups, and differences between groups at each time point, a repeated-measures analysis was performed for cortisol and NKCC.

Fig. 1. NKCC by group over time. ■ = patients; □ = comparison subjects.

In this study, multiple measures were obtained from each individual. Measures within an individual are correlated, but measures between individuals are independent. A typical linear model was inappropriate here because independence of observations would be violated. Therefore, for these analyses, we used a linear model using restricted maximum-likelihood estimation and an unstructured variance-covariance structure. This type of analysis is appropriate for data that are correlated and is performed using methods of mixed linear models. Tests of individual time points can be obtained from these models. Those corresponding test statistics follow a \( t \) distribution and are presented. Because observations were obtained for all subjects at the same measurement times and because of the non-linearity of the response profiles, time was treated as discrete in all models. Each model controlled for baseline values. All post hoc statistics were adjusted for the number of comparisons.

Pearson correlations were used to examine the relationship between pain, stress, and immunological measures. Discriminant function analysis was performed to determine the predictability of pain, stress, and immune measures between those who had illness episodes during the second week and those who did not. To obtain the discriminant function, variables entered in the model were derived from previously published research (eg, Kiecolt-Glaser et al., Refs. 1, 3, 4, 21) and univariate analyses. \( t \) tests were used to compare chronic stress scores (from the Perceived Stress Scale) between the two groups. All statistical tests were performed using the SAS version 7 statistical package for the PC (45).

Three patients had missing NKCC data for the last two time points. Blood for two patients was hemolyzed from the second and third draw, and for one patient there was inadequate blood to assay at two time points. Baseline blood from three patients was unusable. In one case the baseline blood was hemolyzed, and for two patients the IV catheter was not successfully placed on the first attempt.

**RESULTS**

**Study Sample**

Forty-seven patients and subjects (20 men and 27 women) met the selection criteria and participated in this study. Thirty-three patients (13 men and 20 women) received RCT, and 14 subjects (7 men and 7 women) formed the comparison group. The average age was 41 years (range, 21–66 years). There were no significant age differences between the comparison group and the RCT group, nor were there differences in number of infectious disease symptoms in the prior 2 weeks.

The sample was largely made up of dental subjects who reported low levels of dental anxiety (32). According to our previously published research, 17 of 33 patients met the criteria for low dental anxiety (30). There were no statistically significant differences between patients and comparison subjects (\( p = .21 \)) in scores on the Perceived Stress Scale, and scores were within previously published ranges (patients: mean = 36.9, SD = 6.6; comparison subjects: mean = 34.0, SD = 5.9) (37).

**Manipulation Check**

It was important to confirm that patients and comparison subjects did not differ in the level of engagement while watching the video before RCT. Using a 100-point visual analog scale anchored with “didn’t watch intently” and “watched intently,” subjects were asked to what extent they watched the video. Results showed that there were no significant differences between the two groups on their level of engagement (\( p > .4 \)). To further verify that there were no systematic differences between the two groups in their attentiveness to the videos, all patients and subjects were asked to answer questions about the videos they watched. At least 98% of the questions were correctly answered, with the lowest percentage being 95% and the highest being 100% correct, suggesting no significant differences between groups in attention to videos.

**NK Cell Activity**

Analysis of variance showed that there were no significant differences between patients and comparison subjects at baseline on NKCC (\( p > .99 \)), suggesting that the groups were equivalent at the start of the study. The repeated-measures analysis showed that there was a statistically significant main effect of time (\( F(2,38) = 14.91, p < .0001 \)) and an interaction between time and group (\( F(2,38) = 8.59, p < .0001 \)). There were no significant differences between the two groups at any of the three time points. Within-group analyses revealed a significant increase in NKCC between baseline and 30 minutes after injection in the patient group (adjusted \( p < .0001 \)) and a significant decrease in NKCC among patients between 30 minutes after injection and 30 minutes after RCT (adjusted \( p < .001 \)). It is noteworthy that 55% of patients were below their baseline levels of NKCC at the third time point (30 minutes after RCT). Among the comparison group, there was a marginally significant increase in NKCC from time 1 to time 3 (adjusted \( p = .07 \)) (see Figure 1).

**Cortisol**

For the entire sample, there was a marginally significant correlation between cortisol levels at baseline and scores on the Perceived Stress Scale (\( r = 0.30 \)) (36) (see Table 1). The repeated-measures analysis showed there was not a significant group-by-time interaction effect for cortisol. However, there was a statistically significant main effect of time (\( F(2,41) = 36.90, p < .01 \)) but not group. Specifically, all subjects had significantly lower levels of cortisol at the third blood draw than at the baseline blood draw (\( p < .0001 \)).
Relationships Among Pain, Stress, and Physiological Measures

Pearson correlations were used to determine relationships among levels of stress, pain, and physiological measures among patients (see Table 1). Greater stress at baseline (visual analog scale) was associated with higher baseline cortisol levels ($r = 0.47$, $p < .01$). The stress during RCT was significantly correlated with pain levels 2 and 6 hours after RCT ($r = 0.79$, $p < .01$ and $r = 0.45$, $p < .05$, respectively).

Patients reporting higher levels of pain at baseline had lower NKCC both concurrently ($r = -0.52$, $p < .01$) and after RCT ($r = -0.44$, $p < .05$). Patients reporting greater pain during RCT had lower levels of NKCC ($r = -0.48$, $p < .01$) 30 minutes after RCT. Pain levels 2 and 6 hours after RCT were also negatively correlated with the final NKCC ($r = -0.43$, $p < .05$ and $r = -0.44$, $p < .05$, respectively).

Illnesses

An important question in this study was whether the stress, pain, and immune changes observed as a result of RCT served as risk factors for the development of infectious illness symptoms during the subsequent 4 weeks. To test this question, comparisons were made between the groups on self-reported illness symptoms and episodes.

There was a significant difference between dental patients and the comparison group in the number of self-reported illness symptoms as recorded in the diary during the 4-week period after RCT (see Table 2). Examining the total diary illness data, we observed that the differences between the two groups occurred primarily during the second week after RCT, when the patient group reported more illness episodes than the comparison group. There were no significant differences between groups in self-reported illness episodes during the first, third, or fourth weeks.

Thirteen of the patients developed an illness episode during the second week after RCT, compared with only 2 subjects in the comparison group. Given this distribution of episodes, further analyses were done within the patient group to establish potential predictor variables of illness episodes at the second week.

Patients who had self-reported illness episodes during the second week had significantly higher pain scores 6 hours after RCT (illness group: mean = 25.2, SD = 24.1; nonillness group: mean = 10.1, SD = 10.9; $F(1,32) = 4.37$, $p < .05$) and more stress during their dental appointment (illness: mean = 35.07, SD = 26.5; nonillness: mean = 12.7, SD = 18.8; $F(1,32) = 8.12$, $p < .05$). There was also a trend toward greater pain 2 hours after RCT in the illness group ($p = .06$). NK activity 30 minutes after RCT was significantly lower among individuals who later reported illness episodes than those who did not (illness: mean = 108.7 lytic units, SD = 82.30; nonillness: mean = 209.9 lytic units, SD = 176.3; $F(1,32) = 5.34$, $p < .05$). There was a trend toward higher cortisol levels at the final blood draw in the illness group as compared with the non-

### TABLE 1. Correlations Among Main Study Variables

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>During RCT</th>
<th>After RCT</th>
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</thead>
<tbody>
<tr>
<td>Total sample ($N = 47^a$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic stress</td>
<td>0.30*</td>
<td>0.17</td>
<td>0.24</td>
</tr>
<tr>
<td>Patients only ($N = 33$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anticipated pain</td>
<td>0.33</td>
<td>0.22</td>
<td>0.46*</td>
</tr>
<tr>
<td>Anticipated unpleasantness</td>
<td>0.21</td>
<td>0.12</td>
<td>0.42*</td>
</tr>
<tr>
<td>Pain before RCT (baseline)</td>
<td>-0.12</td>
<td>-0.15</td>
<td>-0.02</td>
</tr>
<tr>
<td>Stress before RCT (baseline)</td>
<td>0.47*</td>
<td>0.27</td>
<td>0.26</td>
</tr>
<tr>
<td>Pain during RCT</td>
<td>-0.14</td>
<td>-0.1</td>
<td>0.23</td>
</tr>
<tr>
<td>Unpleasantness during RCT</td>
<td>-0.02</td>
<td>-0.03</td>
<td>0.33</td>
</tr>
<tr>
<td>Stress during RCT</td>
<td>0.22</td>
<td>0.16</td>
<td>0.32</td>
</tr>
<tr>
<td>Pain 2 h after RCT</td>
<td>-0.17</td>
<td>-0.22</td>
<td>0.16</td>
</tr>
</tbody>
</table>

*a Equivalent time points for patient and comparison groups.
*p $< .05$.

### TABLE 2. Mean Illness Symptoms and Episodes Between Dental Patients and Comparison Subjects

<table>
<thead>
<tr>
<th></th>
<th>Dental Patients ($N = 33$)</th>
<th>Comparison Subjects ($N = 14$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total illness symptoms (SD)*</td>
<td>35.87 (44.6)</td>
<td>7.14 (12.0)</td>
</tr>
<tr>
<td>Illness episodes during second week (SD)*</td>
<td>0.4</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*a Wilcoxon rank sum, 7.37; $p = .007$.
*b Wilcoxon rank sum, 4.78; $p = .03$.
illness group (illness: mean = 14.89, SD = 7.1; no illness: mean = 11.15, SD = 3.9; \( F(1,41) = 3.17, p = .09 \)). It should be noted that a review of the dental charts showed that the two groups did not differ on pulpal or periapical diagnosis. For instance, there were no more cases of irreversible pulpitis among the illness group than among the nonillness group.

Classification of Illness

To further understand the relationship between the risk factors and self-reported illness episodes, discriminant function analysis was used to classify patients into groups of those who reported an illness episode and those who reported no illness episode during the second week after RCT. Based on the univariate analysis and variables identified in prior studies (eg, Herbert and Cohen, Ref. 14), the following variables were entered into the discriminant function: pain 2 and 6 hours after RCT, stress experienced during RCT, and NKCC 30 minutes after RCT. Variables that failed to achieve a probability of <.15 were eliminated from the model. Subjects who did not have complete data were excluded from the analysis.

The final function included pain 2 hours after RCT, the level of stress reported during RCT, and NKCC 30 minutes after RCT. The overall model was statistically significant (\( F(3,21) = 8.23, p < .001 \)) and classified all but three subjects into the correct category (see Table 3). The following variables provided significant independent contributions to the final model: stress experienced during RCT (\( F(1,21) = 23.62, p < .001 \)), pain 2 hours after RCT (\( F(1,21) = 15.93, p < .001 \)), and NKCC level 30 minutes after RCT (\( F(1,21) = 6.84, p < .05 \)).

**TABLE 3. Discriminant Function Analysis\(^a\) Classifying Subjects With and Without Illness 2 Weeks After RCT Using Pain 2 Hours After RCT, Self-Reported Stress During RCT, and NKCC as Predictor Variables**

<table>
<thead>
<tr>
<th>Week 2</th>
<th>Model Classification</th>
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<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Actual by Category</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Actual presence, no illness episode</td>
<td></td>
</tr>
<tr>
<td>Actual presence, illness episode</td>
<td></td>
</tr>
<tr>
<td>Total classified into category by model</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>14</td>
<td>93</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>16</td>
<td>64</td>
</tr>
</tbody>
</table>

\(^a\) \( F(3,21) = 8.23, p < .001 \).

**DISCUSSION**

This study used a naturalistic stressor, RCT, to test the relationships among pain, stress, NKCC, and post-RCT illness vulnerability. The main findings of this study were that the patient group developed significantly more self-reported illness episodes 2 weeks after the procedure than the comparison group and that the predictor variables of NKCC after treatment, stress, and pain correctly classified 88% of the patients into a category of illness episode or no illness episode. Given that millions of similarly stressful and painful medical/dental procedures are done each year, these findings are particularly relevant for patients who may be vulnerable to complications or negative health outcomes.

In addition, we observed a negative relationship between self-reported pain and NKCC at multiple time points, including higher baseline pain and lower concurrent NKCC and lower NKCC 30 minutes after RCT. Patients who reported sustained high levels of pain 2 and 6 hours after treatment were also those who had lower NKCC 30 minutes after treatment. This study is one of the first prospective human studies to show that pain may be associated with suppression of NK cell activity. In contrast to the statistically significant negative relationships between NKCC and pain, there was not a statistically significant relationship between NKCC and self-reported stress during RCT.

**Effects of RCT on NKCC and Cortisol**

As shown in Figure 1, the NKCC response pattern between and within the groups across the three blood draws is complex, and there is large variability within the groups at any given time point. Prior research has shown that patients find RCT to be highly stressful and categorize it along with oral surgery treatment as producing the highest level of dental stress (46). Patients also frequently report that RCT is painful (30). Changes in NKCC in response to stress (21) and pain (25) have been previously reported. During treatment, as shown in Figure 1, patients had greater NKCC 30 minutes after injection than at baseline or after RCT. It is probable that the changes in NKCC we observed are a result of the treatment (RCT). The changes may be due to the stress, due to the dental injection, related to the short-term effect of the local anesthetic, or a combination of these. This pattern is consistent with those found by Greisen et al. (24), who observed a slight increase in short-term NKCC after acute pain stimuli. This increase was blocked when local anesthetic was administered before the pain stimuli, suggesting that acute pain produced the increase. On the other hand, in this
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study we observed negative correlations between self-reported pain and NK cell activity at multiple time points. This latter finding is consistent with reports of Liebeskind (11), Kiecolt-Glaser et al. (23), and Page et al. (10) showing immunosuppression in response to pain.

The mechanism by which pain may disrupt the equilibrium of the immune system has yet to be fully described. One proposed mechanism is glucocorticoid-induced immune suppression. However, consistent with Page et al. (25), we did not find support for a mediational role of cortisol between pain and NKCC.

It is now believed that the adrenergic effects on NK cells consist of two components: first, a large transitory increase in NK cells leading to an increase in activity per unit of blood, followed by a suppression of activity at about 50 minutes after the stressor (47). Consistent with this explanation, we observed an overall pattern that shows an initial rise 30 minutes after dental injection followed by a decrease 30 minutes after RCT. The reader is reminded, however, that Shakhar and Ben-Eliyahu’s (47) study was an animal study, so generalizations may not apply to humans. On the other hand, 55% of the patients showed a decrease in NKCC below their baseline at this third blood draw, which was approximately 1 ½ hours after the injection. Not only is this pattern consistent with that predicted by Shakhar and Ben-Eliyahu, but this drop may parallel the drop observed by Benshop et al. (22), who observed that the acute rise of NKCC after a stressor was followed by a sharp decrease. NKCC suppression after surgical stress reaches its nadir at 24 hours, suggesting that following the recovery pattern longer than 30 minutes after RCT may reveal important patterns of post-stressor NKCC dynamics (48). Whether this suppression has a long-term impact on overall vulnerability to disease remains to be determined, but the illness data from this study suggest that it may.

It should be noted that the trend among the comparison group toward greater NKCC at the final blood draw as compared with baseline (p = .07) is consistent with other research showing a rise in NK cell activity after planned relaxation interventions (49, 50). It is unclear, however, whether passively watching videos would produce the same effect as progressive muscle relaxation, focused breathing, or imagery on immune function, but the possibility cannot be ruled out.

Pain, Stress, and Illness

Patients reported more illness symptoms during the entire follow-up period and more illness episodes during the second week than comparison subjects. Among those who became ill, reported stress level during RCT and pain levels after RCT were significantly higher than among those who did not become ill. In addition, NKCC of patients who developed an illness episode was significantly lower after treatment than among those who did not develop an illness. Although admittedly speculative, the development of illness 2 weeks after RCT in this study may point to the diminished capability of NK cells among those who had experienced the greatest RCT pain. The data we present here suggest a relationship between acute changes in NKCC and the development of upper respiratory tract infection; however, in the absence of medical documentation of reported illness, this conclusion remains tentative. In addition, collection of a more detailed history of upper respiratory symptoms in the period before the study would have helped to rule out other health-related factors that might have affected illness vulnerability.

The discriminant function model showed that higher levels of pain and acute stress, along with lower NKCC, were associated with the presence of a self-reported illness episode during the second week. It has been previously shown that stress and upper respiratory illness are associated (51), and there are published data showing a link between pain and suppression of NKCC (25). This prospective suggests a link in humans among pain, suppression of NKCC, and the development of cold and flu symptoms. Readers should, however, interpret the results of the discriminant function cautiously because of the relatively low subjects-to-variable ratio (52). If these data hold in future investigations, they will provide additional evidence that effective management of pain and stress are important considerations to ensure the well-being of the patient both immediately after and in the weeks that follow medical interventions.

Limitations

One alternate interpretation of these data is that the disease state that underlies the need for RCT may produce an impairment of the immune system and may be the major contributing factor to the differences in NKCC and the observed illness symptoms. Although this is plausible, there are several factors that suggest otherwise. There were no significant differences in baseline NKCC between patients and comparison subjects. The dental diagnoses between those patients who became ill and those who did not were not different. Even if there were differences in the etiology of the dental disease that we did not identify, no relationship has been reported between bacterial infections of odontogenic origin (ie, periapical endodontic infections) and systemic disease states other than in-
flictions states resulting from the acute uncontrolled spread of the initial bacterial infection (53). Nevertheless, bacteria have been shown to alter the immune host response, and we cannot rule out the possibility that our results are related to the oral disease underlying the need for RCT. In addition, strict asepsis was practiced in the study clinic. These aseptic procedures make it unlikely that patients were exposed to microbes during treatment that could account for an increase in infectious disease symptoms. On the other hand, with the present design we cannot rule out the possibility that some factor related to the RCT may have increased patient’s vulnerability to illness. In retrospect it would have been useful to include a “non-operated” comparison group. In fact, it is a challenge to find any dental procedure that is not invasive because even cleaning requires “probing” of the soft tissue, frequently without the benefit of local anesthetic. This probing often causes bleeding, and the process is described by some patients as being as painful as having fillings placed (29). Without flow cytometry data for the number of NK cells, we cannot rule out the possibility that the alteration in NK cell activity seen in the RCT group may have been somewhat influenced by changes in NK cell numbers. However, as Shakhar and Ben-Eliyahu (47) have demonstrated, in vivo β-adrenergic stimulation suppresses NK activity independent of the transitory increase in peripheral NK numbers. Additionally, Z. K. Ballas (University of Iowa, unpublished observations, 1997) reports no significant correlation between NK cell number and NK activity (54). Thus, it is unlikely that the alterations we observed are purely reducible to changes in cell migration patterns, but that possibility cannot be ruled out.

Second, pain, stress, and illness variables are based on self-reports. Although illness data were elicited by a nurse and checked carefully with interview probes, these data may reflect a subject’s tendency to overreport pain, stress, and illness symptoms (55). It is also possible that a propensity toward a negative report bias may be a contributing factor in the low NKCC observed in such individuals (56, 57). Medical documentation of the illness symptoms would have strengthened the reported relationships. However, the relationships of the pain and stress reports with the biological measures in a prospective study provide some objective corroboration of their validity.

**SUMMARY**

This study examined the effects of an acute stressor (RCT) on NKCC and the subsequent development of symptoms of upper respiratory illness during the following 4 weeks. Patients showed greater overall illness symptoms and illness episodes in the second week after RCT than comparison subjects. Discriminant function analysis showed that a combination of elevated stress during RCT coupled with a depressed NKCC and elevated pain after RCT resulted in the correct assignment of 88% of the patients into the correct illness episode category. This finding suggests that even limited transitory changes in immunologic equilibria may be of consequence to health (14). The RCT model provides an innovative paradigm in which to pursue mechanisms that may underlie the recovery period following exposure to pain and stress and subsequent health consequences.

**REFERENCES**

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