Immune Activation and Depression in Women with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** We examined markers of immune activation during periods of stress and depressive symptoms in 45 female patients with rheumatoid arthritis (RA) in comparison to 106 controls with no autoimmune disease.

Methods. Depressive symptoms were recorded, clinician ratings of disease activity were made, and blood was drawn for RA patients and controls at baseline and during a designated stressful week.

Results. Counts of T cell subpopulations revealed significant differences between RA and control groups in proportions of CD8 and CD4 cells, with higher CD4 and lower CD8 counts for the RA participants. Significant depression by diagnosis interactions were found, revealing greater CD4 activation among RA patients who were depressed in comparison to other groups. Only marginally significant effects of stress were found on T cell counts. Interleukin 6 (IL-6) concentrations also differentiated groups, with the highest levels of IL-6 observed for depressed RA patients under stress.

Conclusion. These findings provide new evidence that psychosocial factors play a significant role in autoimmune processes that underlie RA. (J Rheumatol 2004;31:457–63)

Key Indexing Terms:
AUTOIMMUNITY DEPRESSION STRESS
RHEUMATOID ARTHRITIS OSTEOARTHRITIS

A number of immune abnormalities differentiate rheumatoid arthritis (RA) from other forms of arthritis. Consistent with immune activation, peripheral blood measures in patients with RA show elevations of T helper cell (CD4) populations, decreases of T suppressor cells (CD8), and increased circulating concentrations of C-reactive protein, soluble interleukin 2 receptor (sIL-2R)[1,2] and the proinflammatory cytokines IL-6 and IL-1 compared to samples drawn from subjects who do not have RA[3,4]. Despite the salient role of immune activation in RA, there is considerable variability in these immune measures between RA patients, and the mechanisms that account for individual differences of immune activation and disease progression are not fully understood. This study examined differences in immune function between an RA sample and comparable groups of patients with osteoarthritis (OA) and healthy controls, and tested whether psychosocial factors would account for some of the individual differences in immune response observed.

Interpersonal stress and psychological depression are increasingly implicated as psychosocial factors that contribute to neuroimmune dysregulation and severity of disease activity in RA. Stressful events, particularly those of an interpersonal nature, provoke symptoms of disease such as greater pain and functional limitation[5,6]. While less is known about the effects of psychological stress on neuroimmune processes in RA, acute stress and major depressive disorder have been associated with variability in CD8 cells[7] and elevated circulating levels of IL-6[8-10] and sIL-2R[11,12] in non-RA subjects. Further, in studies of RA patients, interpersonal stress and depression have been associated with greater pain[13], and variations in stress and negative mood were found to correlate with immune activation[14-16].

In sum, recent research supports a biopsychosocial model for disease progression in RA. Two psychosocial factors, interpersonal stress and depression, appear to contribute to immune activation and inflammation among RA patients. We examined the effects of psychological stress and depression on markers of immune activation, and the relevance of these measures in predicting disease activity in RA. We predicted that RA participants would show higher concentrations of circulating IL-6, greater numbers of T helper cells, particularly activated T helper cells, and lower numbers of T suppressor cells than healthy controls and patients with OA. We also hypothesized that interpersonal stress and depression would increase levels of proinflamma-

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tory immune processes in study participants, making these variables risk factors for disease activity in RA.

MATERIALS AND METHODS

Participants. The sample consisted of 151 women who had either RA (n = 45) or OA (n = 51) or were healthy controls (n = 55), and who met study criteria as described below. They were selected from a larger study of 99 RA participants, 101 OA, and 100 controls[13]. All participants were between the ages of 42 and 75 years (mean 61.83, SD 7.17). The majority of participants were married (57.9%), 94% were Caucasian, and 36% had completed 4 years of college. Only postmenopausal women were studied to reduce within- and between-group heterogeneity in the hormonal profiles that might underlie stress-related inflammatory processes. In addition, patients were excluded if they had taken D-penicillamine, imuran, gold, nitrogen mustard, or any experimental immune-modifying medications (e.g., monoclonal antibodies) within the past 6 months, or if they had ever taken cytotoxin (or cyclophosphamide). Patients were allowed to be taking modest dosages of methotrexate (MTX; ≤ 10 mg) and oral prednisone (≤ 7.5 mg), and supplementary analyses were conducted on the possible influence of these medications on the findings. Patients were required to be taking stable dosages of these medications for 2 months prior to enrollment. If a patient received a steroid injection, her participation in clinician assessments was suspended for one month, to reduce any effects that this medication may have had on immune variables.

Participants were recruited in a variety of ways including newspaper ads, mailings to Arthritis Foundation members, and through rheumatology clinics. All RA and OA patients had their diagnoses confirmed by their rheumatologists, and then were reconfirmed as meeting American College of Rheumatology criteria by one of the principal investigators (DCY) based on comprehensive reviews of medical history, medication use, and joint tenderness and swelling evaluations made during the in-home visits[17]. To insure that patients were not asymptomatic, only those RA and OA patients who rated their average pain at least 30 on a scale of 0–100 (0 = no pain to 100 = pain as bad as it could be) and who reported moderate or greater activity limitation on the Health Assessment Questionnaire (score ≥ 0.5[18]) were enrolled. All participants were paid $50 initially and another $50 after they completed the study. The Institutional Review Board at Arizona State University approved the study protocol.

Procedure. The data used for this study came from 2 in-home visits by nurse clinicians, an initial mailed questionnaire, and 12 to 20 weekly telephone interviews. The initial questionnaire contained a measure of depressive symptoms. The weekly interviews were conducted by trained research assistants and included measures of interpersonal stress and self-reported arthritis disease activity. These measures were used to determine a week of low disease activity and low interpersonal stress (baseline) and a week of high interpersonal stress (stress). Participation was discontinued after 12 weeks if they met the criteria for baseline and stress weeks. If they had not met these criteria by 12 weeks, they continued in the study for up to 8 additional weeks until they met criteria. (Only the 151 participants with valid baseline and stressful weeks were included in this study.) The in-home visits were conducted within 36 hours of designating the baseline and stress weeks and included a clinician's global assessment of disease activity, a measure of depressive symptoms, and a blood draw used to assess immune function. These assessments were conducted between 8 AM and 10 AM for all participants to stabilize the influence of circadian rhythms on immune variables.

Table 1 shows analyses comparing the RA sample with OA and control groups on demographic conditions and depression at baseline and during a stress week. Groups were alike on all demographic indices with no significant differences between RA and non-RA in depressive symptoms at baseline or during a stressful week.

Measures. Depressive symptoms. The measure for depressive symptoms consisted of 9 items taken from the *Mental Health Inventory*[19]. The items included questions such as, "How often have you felt like crying?" and "How much of the time have you felt downhearted and blue?" Participants were asked to respond to each of these items with regard to the previous week. The responses were scored on a 6-point scale for all items, except for the last, which was scored on a 5-point scale. Cronbach's alpha was > 0.90 for all participant groups.

Criteria for baseline and stress week. Baseline week. Participants needed to report no increase in disease activity over the prior 2 weeks, and no significant change in interpersonal stress in the past 2 weeks to qualify a week as baseline. A significant change in interpersonal stress (defined below) was designated a "stress week." Disease activity was assessed by self-report each week for the RA and OA participants. To qualify for baseline, the participant could not report significant increases in 2 or more of the following categories using a set of questions validated in prior research[5]: (1) average pain in the past week using a 0–100 numeric rating scale, (2) joint tenderness in 8 joint pairs, (3) self-report of curtailment of normal activities due to arthritis, and (4) self-reported significant increase in overall arthritis activity.

Stress week. The procedure for determining a stressful week involved meeting at least one of 3 criteria. The first criterion was defined solely in terms of perception. A participant was considered to be in a stressful period if they rated any relationship domain (spouse/significant other, family members, friends, coworkers) as being "much more stressful" for that week than the previous week. The second criterion was defined in terms of stressful interpersonal events alone. Stressful interpersonal events were measured each week by 21 items of the Inventory of Small Life Events (ISLE)[20]. Participants were asked to report the number of times each event happened during the past week. These events were assessed in 4 kinds of relationships: spouse or significant others (4 items), family members (2 items), friends (6 items), and coworkers (9 items). Examples of negative events assessed include arguments with spouse, criticized by a family member, meeting unfriendly or rude people, and pressure to work harder or faster. A baseline score on number of negative events was computed as the average of the first 3 weekly reports of negative events. Participants whose total negative interpersonal events score doubled (summed across all domains) from their baseline score, and whose total negative interpersonal events score was ≥ 7, were considered to be in a stressful period. These cutoff scores were determined through analysis of data from previous studies on stressful events[5] in which the ISLE was used to measure interpersonal stress. The third criterion served as a compromise between the first 2 criteria. If a negative interpersonal event score within any of the domains had doubled from a baseline period, and that domain was perceived to be "more stressful," that participant was deemed to be in a stressful period.

Clinician ratings of disease activity. Nursing clinicians trained and supervised by the study rheumatologists provided a global assessment of current disease activity during the baseline and stress weeks, after conducting a joint examination of RA and OA participants. A global assessment has been identified as one of the single best indicators of disease activity[21]. Using a 4-point scale, disease activity was rated as 1 = no flare, 2 = mild flare, 3 = moderate flare, and 4 = severe flare. In prior research, this global measure has shown moderately strong correlations (r > 0.62) with joint tenderness and current pain in arthritis patients[22]. Nurse clinicians were blinded to the condition of the participant at the time of their assessment, but were likely to be able to distinguish RA from OA subjects during their examinations. Since this global rating may have been applied differently for the 2 diagnostic groups, making the measure more valid as an indicator for one group, we examined the correlation between global ratings and a rating of average pain (described below) to provide one test of the comparability of global assessments between groups. For OA subjects, global ratings at stress correlated (r = 0.38) with self-reported pain. For RA subjects, the global ratings correlated (r = 0.42) with pain. Both correlations were significant at $p < 0.01$, and were not significantly different from one another. These data provide assurance that the global ratings were valid indicators of disease activity for both groups. In addition to the global rating of disease activity, clinicians also provided ratings of tenderness using a 4-

Table 1. Demographic characteristics of rheumatoid arthritis (RA) and non-RA groups.

	Non-RA X or % (SD)	RA X or % (SD)	Test Statistic t or chi-square*
Age	62.10 (7.50)	61.20 (6.40)	0.753
Income > 25K	62.7	63.3	0.004*
Married	54.8	66.7	1.29*
College graduate	33.3	43.3	0.95*
Non-white including Hispanic	6.7	4.4	0.289*
No. of children	3.34 (1.94)	3.33 (2.03)	0.010
Depression, baseline	2.01 (0.81)	1.87 (0.78)	0.98
Depression, stress	1.91 (0.70)	2.05 (0.83)	–1.02

All tests of differences between groups nonsignificant (p = 0.10).

point scale (none, complaint, complaint and wincing, wincing and attempt to withdraw) on 34 joints on each side of the body and ratings of lumbar spine and cervical spine. A tenderness score was computed by summing these 70 ratings. This score was used as a secondary rating of change in clinical status from baseline to stress. These ratings were correlated significantly with global ratings for OA and RA groups with r = 0.58 and r = 0.51, respectively. However, the level of correlation of tenderness scores with pain reports (r < 0.27) was lower than that found for global ratings.

Pain. The average level of pain was assessed using the following numeric pain rating scale: "Please choose a number between 0 and 100 that best describes the average level of pain you have experienced over the past week due to your arthritis. A zero (0) would mean 'no pain' and 100 would mean 'pain as bad as it could be'." Scores on this measure were taken on the 2 weeks prior to the stress week and used as a covariate in the analysis of changes in disease activity from the baseline to the stress week. Pain data collected the week of stress were used to probe the validity of the clinician ratings.

Somatic complaints. It was conceivable that patient reports of pain, joint tenderness, and interpersonal stress could reflect individual differences in predisposition to voice general somatic complaints. To control for such potential confounds, all participants were asked to report the degree of discomfort over the past year using a 5-point scale on 10 health related symptoms distinct from arthritis pain (e.g., dizziness, chest pain, blurred vision, shortness of breath, nausea, and abdominal pain). These items were constructed from symptom lists on DSM-IV criteria for somatization diagnosis, and selected from a larger inventory, after factor-analytic work[23]. The scale used in this investigation has been shown to reliably differentiate patients diagnosed with fibromyalgia (FM) from those patients diagnosed with OA[23]. Cronbach's alpha for this study population was 0.73.

Immune measures. Peripheral blood mononuclear cell (PBMC) phenotypes were analyzed at baseline and following a designated stressful week. Primary fluorescein and phycoerythrin labeled monoclonal antibodies (mAb) specific for CD4 (T helper/inducer), CD8 (T suppressor), and CD25 (IL-2R) were used to determine the percentage of single and double-staining PBMC phenotypes. The CD25 readings provided an estimate of T cell activation through measurement of the level of expression of IL-2R on the lymphocytes. This assessment, done for total T cells and also separately for T helper and T suppressor subsets, is highly correlated with levels of sIL-2R in plasma, but is a more direct indicator of immune activation than sIL-2R. Whole heparinized blood (50 µl) was mixed with mAb for 30 min at room temperature and then lysed before being washed 3 times with phosphate buffered saline (PBS) and analyzed in a Becton-Dickinson FacsScan flow cytometer. Data from 10,000 cells were analyzed using a Consort 30 software package (Becton-Dickinson). Serum IL-6 levels were measured in duplicate using a sandwich ELISA (R&D Systems, Minneapolis, MN, USA).

RESULTS

There were 3 factors in the design. One was the repeated measure, baseline versus stress week (week). Depression level was a second factor. Self-reports of depression recorded at baseline and at stress were averaged and participants assigned to high or low depression groups based on whether their average scores on depression were above or at/below the median for the study population. The other factor in the design was diagnosis: RA versus OA and controls. The 2 control groups were combined since differential effects were not predicted for these groups, and both were expected to differ in immune response from the RA group. Preliminary analyses revealed that there were no differences between OA and control groups on immune responses to stress. Thus, the full design was a 2 × 2 × 2 analysis of variance (ANOVA) with one repeated measure.

Before analysis of study hypotheses, a series of t tests were run to explore the possible effects of age, somatic complaints, estrogen replacement therapy (ERT), and antidepressant medication use on the dependent measures taken at baseline and stress. Age and ERT showed no effects on the dependent measures (p > 0.05), and were not controlled for in subsequent analyses. Somatic complaints were associated with greater joint tenderness at baseline (r = 0.26, p < 0.01) and global ratings at stress (r = 0.32, p < 0.01), but not with any other measure. Thus, somatic complaints were included as a covariant only in the analysis of the clinician ratings. Antidepressant medication use was associated with higher scores on average depression, baseline levels of CD25, and activated CD4 cells at baseline (all p < 0.05), and therefore was treated as a covariant in analyses of those variables.

To guard against any influence of changes in disease activity just prior to the stress week on outcomes that could be a source of confounding, a covariate was introduced into the repeated measures ANOVA: average level of pain reported in the 2 weeks prior to the stress week. This covariate had a significant effect on clinician ratings. Patients reporting higher pain in the 2 weeks just prior to the

stress week received higher clinician ratings of disease activity than those patients reporting less pain ($F = 7.188$, $p < 0.01$). This covariate was retained in all subsequent analyses of clinician ratings. It was unrelated to any other outcome ($p > 0.15$) and thus was dropped in estimation of effects in analyses other than those dealing with clinician ratings.

Clinician ratings made at baseline were consistent with the self-report measures; these ratings were low and not significantly different between RA and OA groups. Clinician ratings were not obtained for controls since this group was free of joint pain. The average global rating for disease activity at baseline using a scale from 1 = no flare to 4 = severe flare was 1.40 (SD 0.495) for RA and 1.51 (SD 0.731) for OA participants. The repeated measures analysis of covariance, controlling for pain in prior weeks and somatic complaints at the initial interview, revealed a significant main effect for week [baseline versus stress; $F (1, 91) = 6.36$, $p < 0.02$] due to a significant increase in global ratings of disease activity from baseline to a stress week. In addition, there was a depression by week interaction [$F (1, 91) = 5.22$, $p < 0.03$]. Figure 1 displays the average clinician ratings of disease activity for depressed and nondepressed groups at baseline, and during a subsequent in-home nursing evaluation made during a stressful week, showing higher increases from baseline to stress weeks for depressed arthritis participants than nondepressed participants. Both RA and OA groups showed elevations in clinician global ratings of disease activity from baseline to stress, with no significant difference between them, as evidenced by a nonsignificant diagnosis by week interaction [$F (1, 91) = 2.50$, $p > 0.10$]. The analysis of joint tenderness scores yielded similar results. There was a main effect for week [$F (1, 96) = 5.75$, $p < 0.02$], and a significant depression by week interaction [$F (1, 96) = 4.55$, $p < 0.04$] of the same pattern as shown in Figure 1, and no effect of diagnosis on the tenderness ratings.

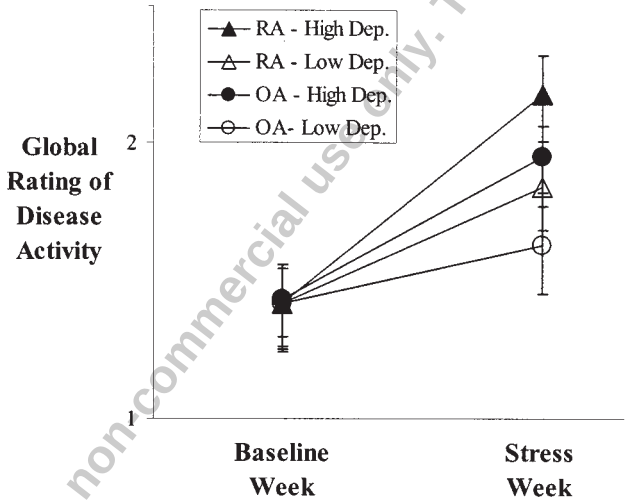

Figure 1. Clinician ratings of disease activity at baseline and a stressful week for RA and OA participants either high or low in depressive symptoms.

The CD4 and CD8 cell counts were subject to repeated measures ANOVA for the 151 participants at baseline and stress weeks. Significant main effects for diagnosis were found: RA patients had greater numbers of CD4 ($p < 0.001$) and fewer CD8 ($p < 0.009$) than non-RA participants. Significant diagnosis by depression interactions further defined these main effects: depressed RA patients showed the highest levels of CD4 cell counts [$F (1, 142) = 5.53$, $p < 0.021$] and the lowest CD8 levels [$F (1, 142) = 3.446$, $p < 0.066$]. Table 2 displays the average scores on immune variables for depressed and nondepressed RA groups and non-RA groups. As Table 2 shows, it was the RA depressed group that showed an immune profile strikingly different from the other groups. There were no significant main effects of week or any interaction effects involving week on T cell counts. However, there was a marginally significant drop in CD8 cells from baseline (X = 22.52, SD 0.91) to stress (X = 22.00, SD 0.87) [$F (1, 142) = 2.82$, $p < 0.095$]. There was also a marginally significant triple interaction [$F (1, 142) = 3.07$, $p < 0.082$] that suggested a modest drop in CD8 counts from baseline to stress for all groups except depressed RA patients who started out with low CD8 counts at baseline (X = 17.15, SD 2.3) and that stayed low at stress (X = 17.27, SD 2.19).

Counts of lymphocytes expressing CD25 were analyzed in the same manner, and also revealed a significant diagnosis by depression interaction [$F (1, 139) = 8.407$, $p < 0.005$]. Similar to the findings for CD4 cell counts, depressed RA patients had the highest CD25 counts (see Table 2). In addition there was a marginally significant Diagnosis by week interaction, $F (1.139) = 3.797$, $p < 0.054$, with a pattern of means suggesting that CD25 cell counts were elevated for RA patients at both baseline (X = 23.05, SD 1.8) and stress (X = 22.10, SD 2.05), but were high for non-RA patients only at stress (X = 21.75, SD 1.36) and not at baseline (X = 19.04, SD 1.19). Followup analyses of 2 subsets of CD25 activation markers on T cells revealed that the activated CD4 cells, and not the activated CD8 cells, were responsible for the findings observed for CD25 cell populations. Table 2 shows these results.

In sum, differences were observed in cell counts between depressed RA patients and other groups, but consistent differences were not found between groups in changes in immune markers from baseline to stressful weeks. The production and release of the proinflammatory cytokine IL-6 plays a key role in the differentiation, maturation, and activation of T cells. Thus, IL-6 assays were conducted on all remaining blood samples. There were 90 participants with viable data remaining at both baseline and stress weeks. Levels of IL-6 were log-transformed prior to analysis and then were subjected to repeated measures ANOVA in the same manner as the other immune markers.

Table 2. Immune cell counts for RA and non-RA groups high or low in depression. Standard errors are provided in parentheses.

	Non-RA		RA	
	Low Depression	High Depression	Low Depression	High Depression
CD4	53.34[b] (1.46)	52.08[b] (1.38)	55.26[b] (2.02)	62.64[a] (2.32)
CD8	24.06[b] (1.39)	25.11[b] (1.32)	22.67[b] (1.93)	17.21[a] (2.22)
CD25	22.84[a] (1.72)	17.95[b] (1.59)	18.88[b] (2.32)	26.26[a] (2.66)
CD25:CD4	12.38[b] (8.29)	12.72[b] (7.40)	14.78[b] (9.37)	20.27[a] (8.88)
CD25:CD8	6.82[a] (11.19)	4.30[a] (6.10)	5.37[a] (6.98)	6.41[a] (5.93)

Row values with the different superscripts are significantly different ($p < 0.05$) from one another.

Overall, RA participants had higher levels of IL-6, as revealed by a main effect for diagnosis [$F(1, 85) = 5.602$, $p < 0.02$]. These differences between RA and other participants were magnified during the stress week, as indicated by a significant diagnosis by week interaction [$F(1, 85) = 4.393$, $p < 0.040$]. There was also evidence that depressed participants showed greater elevations in IL-6 from baseline to stress; the ANOVA results revealed a marginally significant depression by week interaction [$F(1, 85) = 2.876$, $p < 0.095$]. The overall pattern of results is shown in Figure 2. The depressed RA sample at stress showed the highest elevations in IL-6.

Supplementary analyses with subsets of RA participants. There were 13 RA patients taking oral prednisone during this study, and additional analyses were conducted on the RA sample to determine whether patients taking this medication responded differently from those not taking prednisone. Patients taking prednisone reported more depression, higher baseline scores on CD4 activation, and lower levels of IL-6 at stress than RA patients not taking prednisone. Thus, the effects of stress and depression on outcomes were assessed for RA patients in which prednisone use was treated as a factor in the ANOVA design. Two changes in the overall pattern of results were observed in these analyses. First, no change in global ratings of disease activity from baseline to stress was observed for RA patients taking prednisone. Second, the depression by week interaction for IL-6, which was marginally significant before ($p < 0.095$), was now statistically significant ($p < 0.04$), revealing an increase in IL-6 levels from baseline to stress week in depressed RA patients, when the effects of prednisone were accounted for in the ANOVA tests.

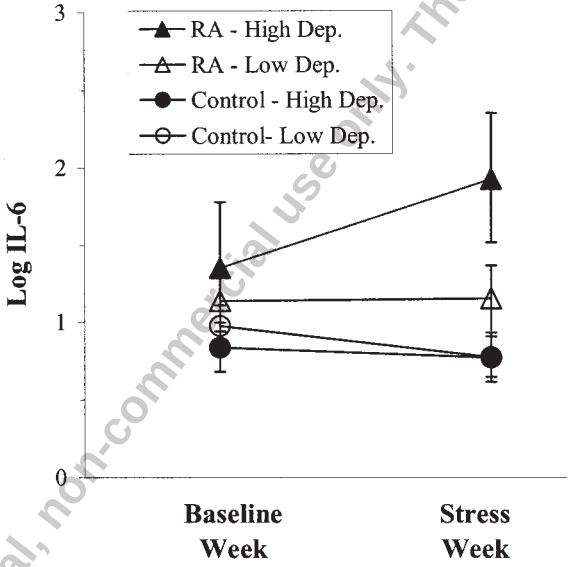

Figure 2. Log IL-6 values at baseline and stress weeks for RA and control groups with low or high depressive symptoms.

There were 32 RA patients taking estrogen replacement therapy (ERT). RA patients using this medication did not differ significantly in clinical ratings of disease activity, on depressive symptoms, or on any of the immune variables tested. When ERT was treated as a factor in the repeated measures ANOVA design, no main effects or interactions were observed for this variable, and there were no changes in the substantive pattern of results as a result of including ERT as a covariate in the design.

There were 22 RA patients taking MTX. Patients using this medication tended to have higher CD4 counts ($F = 5.49$, $p < 0.05$), but there were no other significant relationships with immune measures or with clinical ratings. Treating MTX use as a factor in the ANOVA design when examining CD4 levels did not lead to any changes in the findings: depressive RA participants continued to show significantly higher CD4 cell counts than other groups.

DISCUSSION

Ratings of disease activity were higher during a stress week than during a baseline week, with the depressed OA and RA patients showing the greatest elevations based on the clinicians' ratings of tenderness and global disease activity. These clinical findings are consistent with previous results on self-reported disease activity for the same study patients deduced from analyses of the weekly reports of stress and pain for RA and OA groups[14]. They also set the stage for the examination of immune changes that could account for apparent differences between depressed and nondepressed groups in stress-reactive changes in disease state.

There was consistent evidence that depression amplified the differences between RA and control groups in immune markers, especially for activated CD4 cell counts, and levels of IL-6. Depression was not experimentally induced, raising some doubt about the correct causal order between depression and immune activation. Indeed, higher depression may have followed greater inflammatory activity, either as a consequence of impaired functioning or perhaps directly through the influence of immune changes on affective processes. However, the findings for IL-6 suggested that depression may have served as a risk factor for proinflammatory processes that were elevated during stressful weeks. These results were only marginal in statistical significance, however.

RA patients not only had more proinflammatory cytokines in circulation than non-RA groups, they also showed a greater increase in IL-6 during a stressful week than non-RA subjects. To our knowledge this study is the first to detect such differences between RA and non-RA groups in IL-6 levels in response to life stressors. Our findings suggest that the key risk factors responsible for aberrant inflammatory responses are additive: autoimmune pathology and depression together may increase susceptibility to flares of disease following stress.

It is important to note that the OA patients also showed elevations in clinical ratings of disease activity at stress. Since this study was not designed to detect potential physiological mediators of stress related changes in OA joint tenderness, we can only speculate on the nature of those effects. Pain syndromes such as FM are prevalent in RA and OA populations, and could lead to symptom reports that are unrelated to underlying joint pathology. When our study was conducted, the diagnosis of FM was not given as often or with as much care as is done currently, so we were not confident of the reliability of any reports of this diagnosis. Instead, we relied on a checklist of somatic complaints as a means of estimating tendencies toward somatizing. This measure was correlated with clinician reports of joint tenderness and the clinician's global rating of disease activity, leading us to treat this measure as a covariate in analyses of those variables. Our somatic complaints measure was unrelated to any of the immune measures, which is fairly consistent with the literature. One recent study[24] suggested that cytokine levels might be elevated in FM, including the proliferation of IL-6 in response to antigen stimulation. However, when testing group differences with assays of serum levels of IL-6, as we did in this study, that investigation also did not find higher cytokine levels for patients with FM. Further study of the role of somatization in stress-reactive self-reports of pain and tenderness and accompanying immune processes may clarify these findings. There were few effects of medication use on the dependent measures. RA participants taking MTX or prednisone had somewhat higher CD4 cell counts, and those taking antidepressants were more likely to report depressive symptoms. It seems most likely in those cases that severity of illness is the causal variable for the relationships obtained, and not the medication itself. There may have been more subtle effects on immune measures dependent on dosages that could not be detected here, and some relationships were uncovered that were not expected. Global ratings of disease activity did not increase at stress for the RA subgroups taking oral prednisone, for example. This research was not designed to investigate the effects of medications, and so cannot provide much guidance in the interpretation of these results. In any case, the modest findings for the effects of medication use do support the careful assessment of these variables in future studies of this kind in order to control for additional factors that may influence immune and other systemic physiological processes.

Field studies like this one are valuable in providing a method of examining the role of real-life stressors on changes in disease-relevant indices. They are limited, however, in several important respects. The type of stressor and its magnitude and timing are all outside of experimenter control. Although neither clinicians nor subjects were informed regarding the purpose of each assessment, some clinicians may have discerned the purpose of these examinations. However, we have no reason to believe that their evaluations would be biased based on the level of depression of the participant. Further, we cannot be assured that the clinicians were blind to the diagnosis of the participant. Indeed, we would expect that many would be able to discern the diagnosis from the interview. We may take some measure of confidence that such knowledge did not affect the ratings, in that there were no differences between RA and OA subjects in either global ratings of disease or the tenderness ratings. It is important in this context that the OA sample was selected to be comparable to the RA group on both pain and disability, making them higher in level of illness-related distress than a typical sample of OA patients.

The evidence for overall changes in clinician ratings from baseline to stress should be interpreted with some caution, nonetheless. Baseline clinician assessments were not permitted within 2 weeks of a patient's self-report of a significant increase in disease activity. Although these self-reports were different from the clinician ratings, the study methods may have led to the selection of baseline weeks for which participants had an unusually low level of self-reported disease activity. Therefore, any subsequent assessment may be expected to yield higher scores, regardless of stress level. These method effects may have been responsible for the increases in clinical ratings of disease activity at stress noted for patients with OA seen in Figure 1, without a comparable rise in IL-6 levels (Figure 2). By covarying pain reports from the 2 weeks prior to the stress assessments, we minimized these potential "regression to the mean" effects. Moreover, such regression confounds, if present, would not account for changes observed from base-

line to stress in immune variables, nor could they account for differences found in slope of change in clinical ratings as a function of depression.

Although laboratory settings are not natural contexts for testing the effects of stress, they do provide considerably more control over key factors in the delivery of the stressor and observation of its effects. One of the most important of these controls is the temporal sequence. In this study, stress was assessed over the retrospective week, and blood was drawn up to 36 hours after the report of an elevation in stress. These broad time spans increase error and preclude a careful analysis of biological events that mediate the effects observed. Future research would benefit from the use of a laboratory analog of an interpersonal stressor[25]. This would allow us to study the communication between neuroendocrine and immune regulatory systems when the person is at rest, under stress, and in recovery from stress[26].

These findings led us several steps closer to understanding the role of psychosocial factors in autoimmune disease. Our results indicate that interpersonal stress and depression are intimately related to activated immune processes that promote inflammatory responses. Our findings invite further study of these processes under more controlled laboratory settings. Specifically, we suggest that an examination of proliferative responses of IL-6 as well as other proinflammatory markers to antigen challenge during stressful and nonstressful conditions may offer a more refined test of the role of psychosocial factors in autoimmunity. Similarly, the use of positive and negative mood induction may provide an experimental analog of 2 components of depression to test their effects on autoimmune processes. This work may be extended further to testing the clinical efficacy of cognitive-behavioral interventions designed to reduce depressive responding and improve coping with stress. Such therapies provide a means of examining causal relationships between mind and body more fully in patients with RA, and also may lead to treatments tailored for the individual patient.

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