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Chronic stress and regulation of cellular markers of inflammation in rheumatoid arthritis: Implications for fatigue ☆,☆☆

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Abstract

Objectives. This study examined whether chronic interpersonal stress is associated with cellular markers of inflammation and regulation of these responses by *in vitro* doses of glucocorticoids in rheumatoid arthritis (RA) patients. The association between these markers of inflammation and fatigue was also tested.

Methods. Fifty-eight RA patients completed up to 30 daily ratings of the stressfulness of their interpersonal relations. Interleukin-6 (IL-6) production was analyzed in lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cell cultures with and without varying concentrations of the glucocorticoid hydrocortisone. In addition, plasma levels of IL-6 and C-reactive protein (CRP) were analyzed, and subjective ratings of fatigue and pain were obtained on the day of blood sampling.

Results. Multilevel modeling showed that higher chronic interpersonal stress was associated with greater stimulated IL-6 production ($p < 0.05$) as well as greater resistance to hydrocortisone inhibition of IL-6 production ($p < 0.05$). These relations were not accounted for by demographic factors, body mass index, or steroid medication use. Stimulated production of IL-6, in turn, was associated with greater levels of self-reported fatigue, controlling for pain ($p < 0.05$). Neither chronic stress ratings nor fatigue symptoms were related to plasma levels of IL-6 or CRP ($ps > .05$).

Conclusions. Among RA patients, chronic interpersonal stress is associated with greater stimulated cellular production of IL-6 along with impairments in the capacity of glucocorticoids to inhibit this cellular inflammatory response. Moreover, these findings add to a growing body of data that implicate heightened proinflammatory cytokine activity in those at risk for fatigue symptoms.

Keywords: Stress; Cytokines; Fatigue; Rheumatoid arthritis

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by inflammation of the synovium, with symptoms that include joint pain, stiffness, and fatigue. Fatigue is increasingly recognized as an important factor contributing to quality of life in RA patients, and persistent fatigue is one of the biggest obstacles to optimizing function in these patients (Heller and Shadick, 2007). Progression of RA disease varies considerably from person to person, and is affected by a range of immune, neuroendocrine, and psychosocial factors (Uhlig et al., 2000). Included among the immunological factors implicated as key in RA disease progression is the cellular production of the cytokine interleukin-6 (IL-6; Choy and Panayi, 2001). IL-6 is a sig-

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naling molecule produced primarily by immune cells, and plays an important role in coordinating the acute inflammatory response. By stimulating the production of acute phase proteins, including C-reactive protein (CRP), IL-6 acts to enhance inflammation. Circulating levels of both IL-6 and CRP estimate systemic inflammation (Pearson et al., 2003), and are associated with radiographic evidence of joint destruction in RA patients (Forsblad d'Elia et al., 2003; van Leeuwen MA et al., 1995).

Psychological stress has been identified as a factor that contributes to disease activity in RA patients. In a comprehensive review of 27 independent studies involving over 3000 patients, the stress of minor life events lasting hours to days was associated with increased disease activity among adult RA patients (Herrmann et al., 2000). Although the pathophysiological mechanisms accounting for the association between stress and disease activity in RA are not yet clear, available data point to proinflammatory cytokine activity as a potential mediator. In healthy adults, for example, reports of ongoing stressful circumstances are associated with elevations of *in vivo* markers of systemic inflammation, including increases in circulating levels of IL-6 and CRP (Ranjit et al., 2007). Moreover, brief naturalistic stressors, such as academic examinations, also correlate with increases in stimulated IL-6 production in healthy individuals (Segerstrom and Miller, 2004). Although data in RA patients are limited, they also indicate that exposure to minor naturalistic stressors lasting hours to days is associated with increases in circulating levels of IL-6 (Hirano et al., 2001; Zautra et al., 2004).

The hypothalamic pituitary adrenal axis plays a critical role in the regulation of the inflammatory response, such that the secretion of glucocorticoids in response to stress is thought to counter-regulate increases in inflammatory activity. Cortisol suppresses inflammation, in part, by inhibiting cellular production of pro-inflammatory cytokines, and hence is hypothesized to prevent a prolonged inflammatory response (Raison and Miller, 2003). However, when elevations in cortisol are sustained over time, mononuclear cells may become less responsive to the inhibitory effects of cortisol *in vitro*, which may lead to greater increases in the cellular production of IL-6 and possibly other proinflammatory cytokines (Raison et al., 2006). Indeed, among healthy persons who are exposed to ongoing psychological stress, stimulated IL-6 production shows a greater resistance to the suppressive effects of cortisol *in vitro* (Miller et al., 2002). To our knowledge, no study has examined whether such HPA counter-regulatory cellular mechanisms are associated with changes in IL-6 production among RA patients undergoing chronic psychological stress.

The aim of the current study was to elaborate the role of chronic psychosocial stress in the regulation of cellular markers of inflammation in a community-based sample of RA patients. Consistent with the limited data available, we predicted that chronic daily stress among RA patients would be associated with elevated *in vivo* markers of inflammation, as indexed by circulating levels of IL-6 and CRP. We also hypothesized that chronic stress would be associated with increased stimulated mononuclear cell production of IL-6, and with greater resistance to glucocorticoid inhibition of IL-6 production *in vitro*. Finally, given recent evidence that proinflammatory cytokine activity may play a role in exacerbating symptoms such as fatigue (Dantzer, 2001; Musselman et al., 2001), we explored the associations between *in vivo* and *in vitro* measures of IL-6 production and fatigue symptoms in this sample of RA patients.

2. Methods

2.1. Participants

Participants were 58 adults (35 women and 23 men) with physician-verified RA, recruited from the Phoenix, Arizona metropolitan area via newspaper advertisements, mailings, and physician referrals. The majority of the sample was Caucasian (96%), married/partnered (65%), and unemployed (64%), with a median household income between $30,000 and $39,999.

To be eligible for participation, individuals were required to be at least 18 years of age (mean age = 55, range 23–78 years), could not have a diagnosis of systemic lupus or other inflammatory disorder, and could not be using cyclic hormone replacement therapy. Other health conditions and medication use were not exclusionary. Approximately 55% of the participants indicated that RA was their only chronic health condition, whereas 29% reported having one additional health problem, 11% reported two additional health problems, and 5% reported 3–6 additional health problems. The most common co-morbid conditions reported by participants were lung disease (16%), cardiovascular disease (15%), diabetes (11%), and stroke (7%). The most common medications used by participants were steroids (23%), hormone replacement therapies (23%), antidepressants (18%), and thyroid medications (12%).

2.2. Procedures

After being screened into the study via a phone interview, participants returned an informed consent form by mail along with documents authorizing the research staff to contact their physicians to confirm their RA diagnosis. Once RA diagnosis was confirmed, participants completed (1) self-report measures that included demographic, health, social network, and personality variables; (2) up to 30 consecutive daily diaries that included assessment of interpersonal stress; and (3) a laboratory visit that included measures of inflammatory markers and self-reported pain and fatigue. All procedures were approved by the Institutional Review Boards of Arizona State University and UCLA.

2.2.1. Diary assessment

Participants received a packet of 30 paper diaries and 30 stamped, addressed envelopes, and were trained by study personnel via phone regarding completion of diaries. Each evening prior to retiring, participants completed a diary regarding that day's interpersonal events and the prior night's sleep quality, and placed it in the mail the next morning. Participants received up to $90 for completion of the diaries. The majority of the diary records were returned on time (i.e., 66% post-marked by the next day, 87% by the second day), a compliance rate similar to other mail-based diary studies (e.g., Todd et al., 2003). Over 82% of all participants completed all 30 diaries.

2.2.2. Laboratory visit

Between 1 week and 12 months ($M = 15.72$ weeks, SD = 18.52) following initiation of the diaries, participants attended a laboratory visit that began at 1 p.m. and was conducted at the Phoenix Veterans' Administration Medical Center. Circulating levels of IL-6 and CRP as well as IL-6 regulation were determined from blood samples drawn

between 2 p.m. and 2:30 p.m., following 30 min of sitting quietly. Pain and fatigue assessments occurred immediately following the blood draw. The time lag between the diary assessment and laboratory visit was not correlated with levels of chronic stress, inflammatory markers, or pain and fatigue ratings (all $ps > .40$).

2.3. Measures

2.3.1. Chronic stress

The occurrence and stressfulness of negative life events were recorded in the daily diaries via an abbreviated version of the Inventory of Small Life Events (ISLE; Zautra et al., 1990). Participants rated the extent to which negative events that occurred in each of four interpersonal domains (i.e., spouse/partner, family, friends, and work) were stressful on a scale ranging from 1 (not at all) to 4 (extremely). Individual differences in chronic stress were derived by averaging participants' daily reports of stressfulness across the four interpersonal domains, and across the 30 days of diaries.

We examined the extent to which these chronic stress scores were distinct from indicators of illness severity (i.e., ratings of general health and bodily pain, number of co-morbid health conditions, medication use), general distress (i.e., depressive symptoms and neuroticism), and global social relations (i.e., perceived support and conflict), all assessed via questionnaires at the initial assessment completed upon enrollment in the study. In the current sample, Pearson product-moment and point-biserial correlations indicated that chronic stress scores were positively related to level of depressive symptoms [$r = 0.32$; $p < 0.03$; Mental Health Index (Veit and Ware, 1983; Zautra et al., 1990)], and inversely related to perceived global social support [$r = -0.36$; $p < 0.006$; MOS Social Support Survey (Sherbourne and Stewart, 1991)]. In contrast, chronic stress was unrelated to global perceptions of social conflict [$r = 0.14$, $p < .30$; Negative Social Ties (Finch et al., 1989)], neuroticism [$r = 0.06$–0.08, $p > 0.55$; NEO Five-factor inventory(Costa and McCrae, 1992)], ratings of bodily pain and general health [$rs = 0.04$–0.14, $ps > 0.33$; SF-36 Bodily Pain and General Health subscales (Ware and Sherbourne, 1992)], number of co-morbid health conditions ($r = 0.06$, $p < 0.65$), or use of medications (i.e., steroid, antidepressant, thyroid, and hormone replacement therapies; $rs < -0.08$, $ps > 0.58$). Together, these findings are consistent with the interpretation that chronic stress scores are not a proxy for general distress, global perceptions of social relations, or physical health, but rather reflect individuals' perceptions of ongoing social stress.

2.3.2. Fatigue

A standard 101-point numerical rating scale assessed fatigue following 30 min of rest during the laboratory visit (Jensen et al., 1986; Kirsh et al., 2001). Ratings could range from 0 (no fatigue) to 100 (fatigue as bad as it can be).

2.3.3. Inflammatory markers

Circulating inflammatory molecules. To quantify circulating levels of IL-6 and CRP, 10 ml of blood were collected into EDTA tubes (Becton–Dickinson, Franklin Lakes, NJ), held on ice, and centrifuged within 2 h of collection for 15 min at 1500*g*. Plasma was then aspirated, aliquoted, and frozen at −80 °C until assay. Plasma levels of IL-6 were quantified using Quantikine High Sensitivity human IL-6 kits (R&D Systems, Inc., Minneapolis, MN), an enzyme-linked immunosorbent assay (ELISA) with an intra-assay coefficient of variation of 4% and inter-assay coefficient of variation of 10%. The minimal detectable dose of IL-6 (0.156 pg/ml) is well below levels identified in our subjects. CRP was measured using the Dade Behring N *High Sensitivity* CRP turbidimetric immunoassay (Dade Behring Diagnostics, Marburg, Germany) on the BN ProSpec.

Stimulated IL-6 production assays: To examine the production of IL-6, 10 ml of blood were collected into a heparinized syringe (1 ml), maintained at room temperature, and processed within 3 h of collection. Peripheral blood mononuclear cells (PBMCs) were sedimented on Ficoll–Hypaque (Pharmacia, Piscataway, NJ), washed three times with phosphate buffered saline (Gibco Life Technologies Inc., Grand Island, NY), and resuspended in a 1:1 mixture of RPMI 1640 supplemented with 10% fetal calf serum (Hyclone, Logan, UT; inactivated 1 h in 56 °C water bath), 4 mM glutamine, 20 mM Hepes (Sigma, St. Louis, MO), and 50 mg/ml penicillin and 50 mg/ml streptomycin. Isolated PBMCs (1×10^6 cells per ml) were incubated for 24 h at 37 °C with LPS (100 pg/ml; Sigma Chemical). In addition, to assess the sensitivity of stimulated mononuclear cells to the inhibitory effects of glucocorticoids, PBMCs were co-cultured with LPS (100 pg/ml) along with three concentrations of hydrocortisone (i.e., 10^{-6}, 10^{-7}, 10^{-8} M). Immediately following culture, supernatants were aspirated and stored at −80 °C, and subsequently assayed in batches. Concentrations of IL-6 from the stimulated cell cultures were measured using commercially available ELISA kits (R&D Systems, Minneapolis, MN), which have an intra-assay CV% of 3.1, inter-assay CV% of 2.5, and sensitivity of <0.7 pg/ml. These procedures thus produced four values of IL-6 for each participant, reflecting LPS-stimulated monocyte IL-6 production, and its inhibition by exposure to glucocorticoids. IL-6 values were expressed as ng/ml. All measures of inflammatory markers were skewed, and therefore were log transformed prior to statistical analyses.

Concentrations of LPS and hydrocortisone were determined in a manner consistent with our previously published methods (Bower et al., 2007). Briefly, preliminary studies were conducted to identify a threshold dose of LPS that would produce a reliable increase in the production of IL-6, but that would be substantially below a dose of LPS that induces a maximal level of stimulated cytokine production. We elected to use a threshold dose of LPS, as we and others have shown in animal models that stress can attenuate levels of immune activation when threshold levels of immune stimulation are used, but not when high doses of antigenic or mitogenic activation are applied (Irwin, 1993; Moynihan et al., 1990). Varying concentrations of hydrocortisone were used to capture a range of physiological and supra-physiological glucocorticoid activation, and to yield a linear dose-dependent suppression of stimulated cytokine production with greater than 80% inhibition at the highest concentration (hydrocortisone 10^{-6} M), as demonstrated in preliminary studies of healthy volunteers.

2.3.4. Covariates

Alcohol use was assessed during study screening by asking participants how many alcoholic drinks they consumed during a typical week; scores were then log-transformed prior to analysis. Education level was assessed via a questionnaire item asking participants to indicate their years of education, with response options ranging from 1 (0–4 years) to 8 (postgraduate college). Sleep disturbance was evaluated in the daily diaries with four items modified from the Daily Sleep Diary (Haythornthwaite et al., 1991): participants were asked whether they experienced difficulty falling asleep, staying asleep, awakening too early, or awakening at the time they desired during the previous night/morning. Affirmative responses to items were summed within each day and averaged across the 30 days of diaries to yield a score reflecting individual differences in sleep disturbance. Current pain, BMI, steroid medication use, and systolic and diastolic blood pressure (SBP, DBP) were assessed at the laboratory visit. BMI was derived from assessments of weight and height [BMI: weight (kg)/(height(m)^2)]. Steroid medication use was determined through review of a list of current medications that was provided by participants, coding for use of corticosteroids. Blood pressure was assessed via an IBS-SD-700A automated blood pressure monitor (Industrial and Biomedical Sensors, Waltham, MA) three times during the final 10 min of the 30-min laboratory rest period, and averaged to yield SBP and DBP values. Current pain was assessed on a standard 101-point numerical rating scale (Jensen et al., 1986), with scores ranging from 0 (no pain) to 100 (pain as bad as it can be).

2.3.5. Statistical analysis

The study addressed two primary questions: (1) was chronic stress associated with *in vivo* circulating levels of IL-6 and CRP, stimulated production of IL-6, and *in vitro* suppression of IL-6 production by varying concentrations of hydrocortisone?; and (2) did individual differences in markers of inflammation relate to fatigue in RA patients? For outcomes assessed once (i.e., plasma IL-6 and CRP, and fatigue), the association

Table 1
Means (SDs) of demographic and health-related factors in low and high chronic stress groups[a,b]

Variable	Low stress $n = 29$	High stress $n = 29$	p value for t-test (χ^2)
Age (years)	57.3 (11.3)	53.1 (14.7)	0.26
Sex (% F)	60.7	58.6	(0.54)
Ethnicity (% Caucasian)	96.4	92.9	(0.50)
Education (range 1–8)[c]	5.8 (1.2)	6.1 (1.7)	0.47
Body mass index (kg/m^2)	26.6 (7.0)	29.1 (8.6)	0.25
Systolic blood pressure (mmHg)	120.6 (17.5)	126.1 (20.1)	0.30
Diastolic blood pressure (mmHg)	72.3 (8.0)	76.1 (13.8)	0.24
Sleep disturbance (range 0–4)	1.4 (0.9)	1.5 (0.6)	0.56
Alcohol use (%)	52.4	44.0	(0.39)
Steroid medication use (%)	21.4	24.1	(0.53)
Average pain (range 0–100)	18.2 (23.8)	20.7 (24.7)	0.71

[a] Low/high stress groups based on median split of chronic stress score.
[b] Ns vary between n = 54–58 across variables due to missing data.
[c] Education scores range from 1 = 0–4 years of schooling to 8 = post-graduate degree.

between stress and each outcome was evaluated via Pearson correlations and hierarchical linear regression analyses. Multilevel modeling (MLM) was used to evaluate the LPS-stimulated IL-6 measures, which have a nested hierarchical structure (i.e., four observations nested within each of 58 participants). MLM is able to account for variation both within each person (i.e., Level 1) and between persons (i.e., Level 2), and to control for the correlation between observations from the same individual (i.e., autocorrelation; Blackwell et al., 2006). The multilevel procedures followed the guidelines presented by Singer (1998) to identify the best fitting model of the variances and covariances of the variables under study. In these analyses, intercepts were allowed to vary randomly, and Level 2 predictors were centered on the sample mean. We tested models that included age, sex, ethnicity, BMI, sleep disturbance, alcohol use, steroid medication use, and pain to determine whether any associations between stress and inflammatory markers were accounted for by these factors. All MLM analyses were completed using the SAS PROC MIXED software (Littell et al., 1996), which uses a "state of the art" missing data handling procedure (Schafer and Graham, 2002). For all statistical analyses, the alpha level was set to 0.05 and two-tailed tests of significance were applied.

3. Results

3.1. Clinical characteristics

In the total sample, the daily chronic stress scores were normally distributed, with a mean of 1.37 (SD = 0.33) and a range of 1–2.59 out of a possible score of 4.0. This average daily score for the stressfulness of interpersonal relations fell between ratings of "not at all" and "a little" stressful. To determine whether any background characteristics were associated with level of stress, the sample was first stratified into two groups using a median split of chronic stress scores. Table 1 shows the sample characteristics and health-related variables for the two chronic stress groups; t-test and χ^2 analyses revealed no significant differences between low and high stress groups for any of the demographic, anthropometric, physiological, or health behavior measures (all $ps > 0.24$). Pearson product-moment and point-biserial correlations also were computed to examine these same associations using the continuous measure of stress scores, and indicated that chronic stress was unrelated to any background characteristic.

3.2. Chronic stress and markers of inflammation

The descriptive statistics and intercorrelations of *in vivo* markers of systemic inflammation, *in vitro* stimulated production of IL-6, and chronic stress are shown in Table 2. Although plasma levels of IL-6 and CRP were significantly related to one another ($r = 0.57$; $p < 0.0001$), both were unrelated to LPS-stimulated IL-6 levels. Consistent with our prediction, chronic stress was positively related to LPS-stimulated production of IL-6 across all concentrations of hydrocortisone ($rs > 0.29$, $ps < 0.03$), indicating that RA patients who had higher levels of daily interpersonal stress were more likely to show increases in the cellular production of IL-6. Measures of chronic stress were not related to plasma levels of IL-6 or CRP ($ps > 0.14$).

Multilevel analyses were conducted to further elaborate the relation between chronic stress and IL-6 production by taking into account varying doses of hydrocortisone and its suppression of IL-6 production. In addition, these analyses evaluated the contribution of potential covariates (i.e., age, sex, race, BMI sleep disturbance, alcohol use, pain, and steroid medication use) to the model. Levels of IL-6 were modeled as a function of hydrocortisone dose (Level 1)[1], chronic stress (Level 2), and their interaction. The model also included a first order autoregressive structure [i.e., AR(1)] for Level 1 residuals. Of the potential covariates, only BMI and steroid use contributed significantly to the prediction of IL-6 production and were retained in the analysis.

Results derived from the final model, shown in Table 3, indicated that LPS-stimulated production of IL-6 was diminished as hydrocortisone exposure increased ($p < 0.0001$), as expected. However, the inhibitory effect of glucocorticoid exposure on IL-6 production varied depending on individuals' chronic stress level, as indexed by a significant stress by hydrocortisone interaction

[1] Hydrocortisone exposure was coded as follows: 0 = no hydrocortisone, $0.01 = 10^{-8}$ M, $0.10 = 10^{-7}$ M, and $1.0 = 10^{-6}$ M.

Table 2
Means, standard deviations, and intercorrelations of immune and stress measures[a]

Variable	*M*	SD	2	3	4	5	6	7	8
1. LPS-stimulated IL-6 (ng/ml)	12333.1	8825.4	0.54**	0.50**	0.38**	0.16	0.12	0.29*	0.15
2. LPS-stimulated IL-6 with 10^{-8} M hydrocortisone (ng/ml)	10651.2	6837.2	—	0.92**	0.88**	0.08	−0.05	0.29*	0.19
3. LPS-stimulated IL-6 with 10^{-7} M hydrocortisone (ng/ml)	7536.6	5779.4		—	0.93**	0.00	−0.15	0.39**	0.15
4. LPS-stimulated IL-6 with 10^{-6} M hydrocortisone (ng/ml)	4459.7	3678.7			—	0.01	−0.08	0.40**	0.07
5. Plasma IL-6 (pg/ml)	4.8	4.6				—	0.57**	−0.20	0.12
6. C-reactive protein (pg/ml)	7.7	9.6					—	0.04	−0.12
7. Chronic stress[b]	1.4	0.3						—	0.15
8. Fatigue (0–100)	20.4	24.3							—

[a] $^{*}p < .05$. $^{**}p < .001$. Means and standard deviations are based on untransformed variables for all measures. Correlations are based on natural log transformed values for inflammation variables. Sample sizes vary between 54 and 58 due to missing data on some measures.
[b] Range of chronic stress ratings = 1 (not at all) to 4 (extremely).

Table 3
Multilevel regression predicting LPS-stimulated IL-6 levels (log-transformed) from chronic daily stress and hydrocortisone exposure

Random effects				
Variance–covariance parameter estimates	Coefficient	SE	*Z*	*p*
Intercept	0.31	0.15	2.10	0.02
Autoregressive error, first order	0.67	0.16	4.16	<0.0001
Residual	0.31	0.14	2.12	0.02
Fixed effects				
	Coefficient	SE	*t*	*p*
Predictor Variables				
Within-Person (df = 157)				
Hydrocortisone exposure[a]	−0.87	0.06	−13.51	<0.0001
Between-Person (df = 51)				
Intercept	8.26	0.38	121.72	<0.0001
Chronic stress[b]	0.73	0.30	2.44	0.02
Steroid use (0 = no, 1 = yes)	−0.58	0.23	−2.51	0.02
Body mass index[b]	0.03	0.01	2.37	0.03
Within × Between-Person (df = 157)				
Hydrocortisone exposure × Chronic stress	0.47	0.19	2.45	0.02

[a] Hydrocortisone exposure is coded as follows: 0 = no exposure, 0.01 = 10^{-8} M, 0.10 = 10^{-7} M, 1.00 = 10^{-6} M.
[b] Chronic stress and body mass variables are centered on the sample means.

($p < 0.02$). The interaction is illustrated in Fig. 1, which shows the relation between hydrocortisone dose and IL-6 production for those RA participants with stress scores above (high stress) and below (low stress) the median for the sample. As compared to participants with low chronic stress scores, participants with high chronic stress showed increased levels of IL-6 production overall, along with increases in resistance to the inhibitory effects of the glucocorticoid dose. Moreover, the effects of stress were independent of the negative association of steroid medication use ($p < 0.02$) and the positive association of BMI ($p < 0.03$) with IL-6 production.[2] Comparison of variance–covariance parameter estimates from models with and without chronic stress variables indicated that chronic stress accounted for 7.0% of the between person variation in IL-6 production.

To examine the possibility that the length of the interim period between the assessments of stress and IL-6 accounted for the association between stress and IL-6 production, we repeated the analyses and included a variable representing the interim period (in weeks), and the interaction of interim period with chronic stress in the model. Neither the main effect of interim period nor the interaction of interim period with chronic stress predicted IL-6 production ($ps > 0.70$), whereas chronic stress remained a significant predictor in the model. This suggests that the length of the interval between assessments does not account for the relation between stress and IL-6 production.

3.3. Markers of inflammation and fatigue

Hierarchical regression analyses were conducted to examine the association between inflammatory markers and fatigue, over and above demographic, behavioral, and health-related factors. Because fatigue has been shown to covary with pain in RA (Zautra et al., 2007), pain level was included as a control variable in the models. When

[2] We repeated the analyses including covariates that might account for the relation of chronic stress and IL-6 production, including depressive symptoms, perceived social support scores, and other medication use (i.e., antidepressant, thyroid, and hormone replacement therapies). None of these variables was significantly related to IL-6 levels ($ps > 0.70$), and their inclusion in the model did not alter the findings for chronic stress.

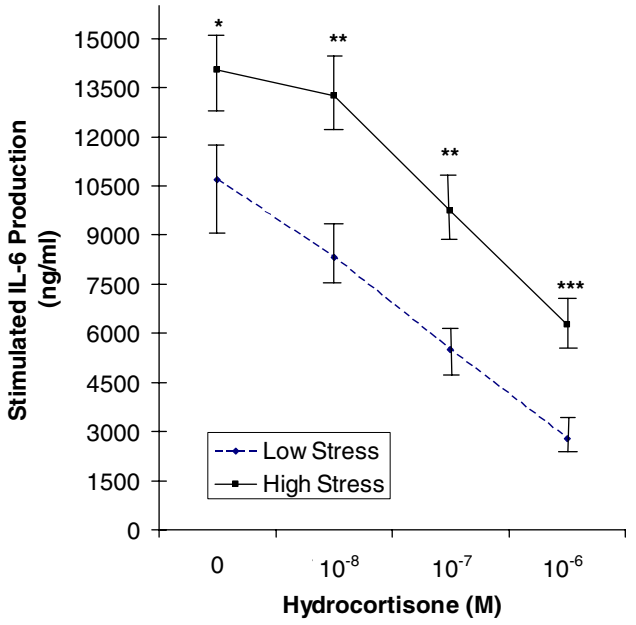

Fig. 1. LPS-stimulated release of IL-6 and inhibition of IL-6 release by hydrocortisone based on chronic stress. Higher chronic stress levels predicted elevated LPS-stimulated production of IL-6 and less suppression of IL-6 production by hydrocortisone. *Note:* Differences based on chronic stress within a concentration of hydrocortisone indicated by $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

pain was included in the initial step of the models, no other covariate (age, BMI, etc) was significantly related to fatigue [$F(1,53) = 40.29$; $R^2 = 0.43$; $b = 0.66$, $p < 0.0001$]. Consistent with our hypotheses, LPS-stimulated IL-6 level contributed to the prediction of fatigue, over and above the contribution of pain [$F(2,52) = 23.58$, $p < 0.0001$; change in $R^2 = .04$; $b = .21$, $p < 0.04$]. In contrast, fatigue was unrelated to plasma levels of both CRP ($r = -0.12$, $p < 0.40$) and IL-6 ($r = 0.11$, $p < 0.43$).

4. Discussion

The current study examined one of the potential mechanisms whereby chronic stress impacts the health of RA patients, through its influence on key aspects of the inflammatory process. We found that chronic interpersonal stress recorded over a 30-day period was associated with both increases in LPS-stimulated IL-6 production by PBMCs, and increases in resistance of PBMCs to the inhibitory effects of glucocorticoid doses. In RA, a condition characterized by excessive inflammation, the chronic stress of social relations may thus contribute to a further shift toward a proinflammatory state. The current findings are the first to our knowledge linking chronic daily stress with increases in IL-6 production in RA patients, and are consistent with findings in healthy adults associating chronic stress with increases in stimulated levels of IL-6 (Miller et al., 2002). The relation of stress to IL-6 production was not accounted for by a number of demographic and health-related factors that have predicted stimulated IL-6 levels in previous research, including age, sex, ethnicity, BMI, steroid use, blood pressure, sleep disturbance, and alcohol use (Marsland et al., 2007; Motivala and Irwin, 2007). Only BMI and steroid use were related to IL-6 production, but including them in the model did not diminish the contribution of chronic stress.

It is particularly noteworthy that in RA patients who reported high vs low levels of stress in their diaries over 30 days, stimulated production of IL-6 was more resistant to the inhibitory effects of hydrocortisone. A number of studies have documented the relationship between cortisol and chronic stress, indicating that more severe, uncontrollable types of chronic stress (e.g., combat, abuse, disaster) are associated with higher daytime levels of cortisol and a flatter diurnal profile (Miller et al., 2007). No consistent HPA profile has been found for RA patients, although it appears clear that cortisol is less effective at inhibiting the cellular production of IL-6 in RA patients who are chronically stressed (Jessop and Harbuz, 2005). Dysregulation may occur at the level of the cellular receptor; for example, female RA patients show a down-regulation in PBMC expression of glucocorticoid receptors compared to healthy controls (Schlaghecke et al., 1994; van Everdingen et al., 2002). The extent to which chronic stress contributes to further down regulation of glucocorticoid receptors on mononuclear cell populations in RA patients requires additional investigation.

Our data suggest that the role of chronic stress in inflammation may have clinical implications in RA; we found that higher LPS-stimulated IL-6 levels related to higher fatigue levels (after controlling for pain). It is important to underscore that stimulated IL-6 production was unrelated to pain levels and it only accounted for an additional 4% of the variance in fatigue once the substantial contribution of pain was taken into account. Nevertheless, the demonstration of a link between the cellular production of IL-6 and fatigue in RA participants adds to prior evidence linking increases in stimulated monocyte production of IL-6 and tumor necrosis factor α with persistent fatigue in breast cancer survivors (Collado-Hidalgo et al., 2006). Although other investigators have observed associations between fatigue and circulating levels of inflammatory markers, such as IL-6 in healthy adults (Papanicolaou et al., 1998), and interleukin-1 receptor antagonist and soluble interleukin-6 receptor in breast cancer patients (Collado-Hidalgo et al., 2006), we found no such associations here. Neither plasma levels of IL-6 nor CRP was associated with fatigue in RA patients.

In contrast to the present observations, other investigators and our research group have reported links between naturalistic stress and elevations in circulating IL-6 levels in RA patients (Hirano et al., 2002; Zautra et al., 2004). The discrepant findings may be due to the relatively low level of disease activity in the current sample, reflected in a mean plasma IL-6 value of 4.66 pg/ml (SD = 4.69). Plasma IL-6 values reported for RA patients by other labs vary considerably, ranging from 7.15 pg/ml (SD = 4.45) to

23 pg/ml (SD = 32) in newly diagnosed untreated RA patients (Crofford et al., 1997; Straub et al., 2002). Plasma IL-6 values in healthy adults, in contrast, are much lower ($M = 2.0$ pg/ml, SD = 1.5; Straub et al., 2002). Thus, plasma IL-6 values observed in the current sample appear to be somewhat lower than those reported in other samples of RA patients, but elevated compared to values observed in healthy controls. A limited range of plasma levels of IL-6 in our sample of RA patients, who show relatively stable disease activity, might have contributed to the failure to detect a statistical association between stress and *in vivo* IL-6 levels.

The lack of correspondence between findings from the current study and those from earlier investigations of RA patients may also be due to differences in the operationalization of stress (Hirano et al., 2001; Zautra et al., 2004). Both earlier reports examined changes in plasma IL-6 related to significant stressors of short duration: for example, in one study, the stress of an impending surgery (Hirano et al., 2001) and in the other study, a week of substantially elevated interpersonal stress (Zautra et al., 2004). In the present study, chronic interpersonal stress was characterized across 30 days, providing an estimate of more sustained, minor daily stress. The mean score for stress in current sample of 1.37 (SD = 0.33) reflected an average daily rating between "not at all" and "a little" stressful, and was comparable to stress scores we have obtained in a similarly aged sample of female Fibromyalgia and osteoarthritis patients ($N = 260$; $M = 1.47$, SD = 0.40). Relatively low levels of chronic interpersonal stress, then, appear unrelated to *in vivo* markers of inflammation but may have ramifications for some aspects of inflammatory processes in RA patients, reflected in *in vitro* IL-6 production. A direct comparison of the relations between shorter term versus prolonged stress and a range inflammatory markers in RA can elaborate the mechanisms linking stress, inflammation, and disease activity.

The lack of an association between circulating levels of IL-6 and stimulated production of IL-6 observed here is consistent with findings reported in two recent studies of healthy adults (Cyranowski et al., 2007; Sjogren et al., 2006). A dissociation between *in vivo* and *in vitro* measures of IL-6 may be due to differences in the sources of the cytokine between assessment methods. Levels of plasma IL-6 in peripheral circulation reflect the cumulative production from a variety of cell types besides PMBCs that are both abundant and widely distributed throughout the body. Sources of circulating IL-6 include endothelial cells, macrophages, T and B cells, fibroblasts, adipocytes, and particularly in RA, synoviocytes and chondrocytes (Heinrich et al., 1990; Ishihara and Hirano, 2002). In addition, all inflammatory markers in the current study were derived at a single time point. Individual differences in LPS-stimulated IL-6 production are highly heritable (de Craen et al., 2005), suggesting that these differences are stable over time. In contrast, circulating levels of CRP and IL-6 are highly sensitive to a variety of stimuli and may require multiple assessments to obtain a reliable estimate of chronic inflammation (Koenig et al., 2003).

Interpretation of the current findings is constrained by several important aspects of the study methodology. First, we employed a sample of patients with RA who were taking a variety of medications for their condition. In analyses predicting IL-6 measures, we controlled for steroid use, a medication regimen prescribed to dampen inflammation. We also explored whether antidepressants, thyroid medication, or hormone replacement therapy were related to inflammatory outcomes, but found no evidence of any significant associations. However, we did not have a large enough sample to systematically evaluate the impact of combinations of classes of medication. Second, we cannot identify the cellular mechanisms responsible for the effects of stress, because we did not assess the cellular composition of the PBMCs. Because LPS primarily stimulates monocytes, IL-6 production was likely due mainly to this population of cells in the samples. However, it is not possible to determine from these data whether chronic stress relates to increased IL-6 production via an increase in the absolute number of monocytes and/or to an increase in the functional capacity of individual monocytes to respond to LPS stimulation. Third, we assessed IL-6, an important pro-inflammatory cytokine but only one of a number of cytokines produced by PBMCs that regulate inflammation. Assessment of profile of cytokines, including those that are anti-inflammatory, would provide a more complete picture of stress-related shifts in inflammatory regulation. Fourth, our assessment of IL-6 production was based on a sample collected at a single time point, which may not provide a valid estimate of inflammatory potential. Although LPS-stimulated IL-6 production appears to be relatively stable (de Craen et al., 2005), multiple assessments of chronic inflammatory markers over time would certainly strengthen confidence in the current findings. Fifth, the time lag between assessment of stress and assessment of markers of inflammation, which varied between 1 week and 1 year, may have limited our ability to detect relations between them, particularly in such a small sample. Finally, because the findings are correlational, we cannot rule out the possibility that factors correlated with both chronic stress and IL-6 regulation account for the findings. Although analyses included key variables related to stress and immune function, including BMI and steroid use, there may be other variables not assessed in the current study that could explain the associations we observed. For example, we did not include assessment of all relevant health behaviors (e.g., dietary factors, smoking, exercise), nor were several of the variables we did include assessed extensively (e.g., alcohol use).

In summary, our data indicate that chronic interpersonal stress is related to both enhanced PMBC production of IL-6 in response to LPS, and to increases in the resistance to glucocorticoid inhibition of IL-6 production in RA patients. Moreover, stimulated IL-6 production is

positively related to reports of fatigue in RA, pointing to its clinical significance. Together, these data suggest one path by which chronic stress contributes to poorer adaptation in RA patients, and highlight the need for longitudinal work examining the long-term clinical ramifications of stress on inflammatory mechanisms in RA.

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