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Stress Activation of Cellular Markers of Inflammation in Rheumatoid Arthritis

Protective Effects of Tumor Necrosis Factor α Antagonists

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Objective. **Psychological stress is thought to aggravate disease activity in rheumatoid arthritis (RA), although the physiologic mechanisms are unclear. Tumor necrosis factor α (TNFα) is an inflammatory cytokine involved in the exacerbation of RA, and TNFα antagonists have emerged as efficacious treatments. The purpose of this study was to determine whether RA patients show increased monocyte production of TNFα following acute psychological stress and whether such responses are abrogated in RA patients taking TNFα antagonists.**

Methods. **A standardized stress task was administered to 3 groups of subjects: RA patients taking TNFα antagonists, RA patients not taking such medications, and healthy controls. Lipopolysaccharide-stimulated monocyte production of inflammatory cytokines was repeatedly measured using intracellular staining and flow cytometry. Subjective stress, cardiovascular responses, adrenocorticotropic hormone (ACTH) levels, and cortisol levels were also measured.**

Results. **The stress task induced increases in subjective stress, cardiovascular activity, and levels of ACTH and cortisol, with similar responses in the 3 groups. In addition, the stress task induced a significant increase ($P < 0.001$) in monocyte production of TNFα among RA patients who were not taking TNFα antagonists. However, monocyte production of TNFα did not change following psychological stress in RA patients taking TNFα antagonists or in healthy controls.**

Conclusion. **Brief psychological stress can trigger increased stimulated monocyte production of TNFα in RA patients. The use of TNFα antagonists protects against stress activation of cellular markers of inflammation in RA patients.**

Psychological stress is thought to aggravate disease activity in rheumatoid arthritis (RA). In 27 independent studies involving ~3,000 RA patients, stress, defined as minor hassles and life events lasting hours or days, is associated with subsequent increases in disease activity (1). Similarly, in animal models of adjuvant arthritis, short-term foot shock stress is associated with increased disease activity and inflammation (2). Exacerbation of RA symptoms is thought to be driven by inflammatory processes, in which tumor necrosis factor α (TNFα) plays a key orchestrating role (3–5). The impact of psychological stress on inflammatory mechanisms in RA has begun to receive attention (6,7), yet no studies have examined whether experimentally induced psychological stress affects TNFα expression in RA.

TNFα regulates a number of inflammatory processes in RA (3–5), such as up-regulating expression of other inflammatory cytokines, including interleukin-1 (IL-1) and IL-6. Expression of TNFα and other inflammatory cytokines in turn promotes a cascade of processes, such as leukocyte infiltration of synovial tissue and increased collagenase and prostaglandin E production, which ultimately leads to cartilage breakdown and

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bone resorption. Hence, blocking the action of TNFα via antagonists is now a major pharmacologic strategy in the treatment of RA. Monocytes are the primary producers of TNFα, and their capacity to produce TNFα can be measured by the ligation of Toll-like receptor 4 (TLR-4) with lipopolysaccharide (LPS). TLR-4 is a primary signaling pathway through which TNFα production is upregulated in RA (8), with high levels of LPS-stimulated monocyte production of TNFα correlating with destruction of cartilage and bone (9). Moreover, TNFα antagonists decrease LPS-stimulated production of TNFα (10).

In this study, we hypothesized that acute, experimentally induced psychological stress would increase TNFα levels, as measured by LPS-stimulated monocyte production, in RA patients not taking TNFα antagonists as compared with healthy controls. Furthermore, we hypothesized that the use of these medications would abrogate stress-related TNFα production. To test this hypothesis, stimulated monocyte production of inflammatory cytokines was examined before and after experimental psychological stress in RA patients taking TNFα antagonists, RA patients not taking these medications, and healthy controls. Given evidence that experimental stress affects the hypothalamic–pituitary–adrenal axis (HPA) and sympathetic responses (11,12), circulating levels of adrenocorticotropic hormone (ACTH) and cortisol, and cardiovascular responses (heart rate, blood pressure, and preejection period [PEP]) were also assessed.

SUBJECTS AND METHODS

Study participants. Twenty-one RA patients (11 taking TNFα antagonists and 10 not taking TNFα antagonists) and 20 age- and sex-matched healthy controls participated in the study. Subjects were recruited through the posting of flyers in UCLA rheumatology clinics and around the UCLA community, as well as through newspaper advertisements. All subjects provided written consent, as approved by the UCLA Institutional Review Board. RA diagnosis was confirmed by board-certified rheumatologists (DK and JF) using the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 criteria (13). Neither RA patients nor healthy controls reported cardiovascular disease, endocrine-related other autoimmune disorders, or acute or chronic infections. None of the subjects was pregnant or taking oral contraceptives. Neither RA patients nor healthy controls had a current psychiatric mood or anxiety disorder, according to the criteria in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.

All RA patients reported being on a stable medication regimen for at least 2 months, including those taking TNFα antagonists. Subjects taking opioid medications and/or $>$10 mg oral steroids were excluded from the study. Subjects taking nonsteroidal antiinflammatory drugs (NSAIDs) abstained from these medications for at least 24 hours before the stress protocol because of the possible effects of these medications on cytokine production. In RA patients, the Disease Activity Score in 28 joints using the C-reactive protein level (DAS28-CRP) (14,15) was used to estimate the extent of disease activity. The DAS28-CRP was calculated from the number of swollen and tender joints, the rheumatologist's estimate of overall disease severity using a visual analog scale, and the CRP level.

Procedures. The study involved 2 visits, an initial eligibility visit and a subsequent stress reactivity visit ~1–2 weeks later. During the eligibility visit, subjects were interviewed by a clinical psychologist (SJM) regarding current psychiatric symptoms using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. Participants provided a medical history and RA patients underwent a physical evaluation, including a 28-joint assessment (DK and JF). For the stress protocol visit, subjects were asked to abstain from alcohol and caffeine use for 24 hours before their scheduled appointment. Subjects arrived at noon and ate a standardized lunch. At ~1:30 PM, subjects were seated in the psychophysiology laboratory for placement of sensors for electrocardiogram (EKG), impedance cardiogram, and blood pressure measurements. Nurses inserted a 21-gauge intravenous catheter into each subject's forearm vein in the nondominant arm. After ~20 minutes of baseline assessment, the stress task was administered, followed by a 60-minute post–stress task recovery period. Subjects were compensated $60 for their participation.

Stress task. The stress task was the Trier Social Stress Task, a standardized laboratory task in which subjects are evaluated on their performance of public speaking and serial subtraction math tasks. The task has been used extensively to induce psychological and physiologic stress, as indicated by increases in self-reported stress, cardiovascular responses, and ACTH and cortisol levels (16). The stress task was composed of the following sections: the speech preparation period (10 minutes), speech delivery (5 minutes), and serial numeric subtractions (5 minutes). After baseline, 2 evaluators entered the laboratory room and informed the subjects about the topic of their speech, which was to discuss their positive and negative traits. Subjects were told that, after this, they would be asked to subtract some numbers for a few minutes. Subjects were told that their speech would be videotaped and evaluated by a panel of experts. The evaluators vocalized a standardized set of statements to heighten the perceived stressfulness of the task (e.g., "Please speak more clearly"; "Please look into the camera"; "Please speak faster").

Self-reported measures. Subjects rated their level of subjective stress on a scale of 0–100, with higher scores reflecting more stress. Ratings were obtained at baseline, immediately after the stress task, and 30 and 60 minutes after the stress task.

Cardiovascular and sympathetic nervous system measures. Blood pressure and heart rate were measured using an automated oscillometric monitor (Dinamap 100; GE Healthcare, Piscataway, NJ). Blood pressure readings were obtained at baseline (i.e., minutes 10, 15, and 19 of baseline), during the stress tasks (i.e., minutes 0, 5, and 9 of speech preparation;

minutes 0, 2, and 4 of speech delivery; and minute 5 of the math task), and after the stress task (i.e., minutes 0, 30, and 60 of recovery). Readings during these periods were averaged. The PEP was measured using an EKG and high-impedance cardiogram (HIC-2000) and COP-WIN software (both from Bio-Impedance Technology, Chapel Hill, NC), using a 4-spot electrode measurement strategy (17). Raw signals were collected continuously during baseline and stress task periods and were synchronized with blood pressure readings during the period after the stress task. Signals were digitized and 60-second ensemble averages were constructed using COP-WIN software. PEP was defined as the time interval, in milliseconds, between the Q wave of the EKG and the B point of the dZ/dt waveforms. Shorter PEP times reflect increased myocardial contractility and increased β-adrenergic sympathetic nervous system drive on the heart.

Blood collection. Blood was collected at 4 times: at the end of baseline, immediately after the stress task, and 30 and 60 minutes after the stress task. Blood was collected into Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ); EDTA tubes were used for subsequent assay of plasma ACTH, cortisol, and IL-6, and heparinized tubes were used for assay of stimulated production of IL-6 and TNFα. Plasma was aliquotted and stored in a freezer at -70°C until assay.

Plasma ACTH and cortisol assays. Plasma levels of ACTH and cortisol were measured using the Advantage chemiluminescence binding assay (Nichols Institute Diagnostics, San Juan Capistrano, CA). For ACTH, the intraassay coefficient of variation (CV) was 2% and the interassay CV was 4%, with a sensitivity of 1 pg/ml. For cortisol, the intraassay CV was 4% and the interassay CV was 6%, with a sensitivity of 5 μg/dl.

Plasma IL-6 and CRP assays. Plasma levels of IL-6 were measured using the Quantikine high-sensitivity human IL-6 kits (R&D Systems, Minneapolis, MN), with an intraassay CV of 4% and an interassay CV of 10%. The minimal detectable dose of IL-6 was 0.156 pg/ml. Plasma levels of CRP were measured using the CardioPhase high-sensitivity CRP assay by means of immunonephelometry (BN II System; Dade Behring, Marburg, Germany). The intraassay CV was 5% and the interassay CV was 6%. The minimal detectable level of CRP was 0.175 mg/liter.

Intracellular production of inflammatory cytokines by stimulated monocytes. Studies in humans show that acute psychological stress increases the overall numbers of leukocytes, including monocytes (18). The extent to which TNFα or IL-6 is produced can vary based on the proportion of monocytes and other leukocytes in the sample. Thus, adequate control of changing cell numbers is essential when assessing stress-related changes in cytokine production. In this study, an intracytoplasmic approach was used, in which TNFα/IL-6 production was assessed on a per cell basis from a standardized number of monocytes.

Monocyte intracellular production of TNFα and IL-6 in unstimulated and LPS-stimulated whole-blood leukocytes was assessed by flow cytometry using peridinin chlorophyll A protein (PerCP)–labeled CD14 monoclonal antibody (mAb), allophycocyanin (APC)–labeled anti-TNFα mAb, and phycoerythrin (PE)–labeled anti–IL-6 mAb, as previously described (19,20). Briefly, heparin-treated blood (1 ml) was mixed with or without 100 pg/ml of LPS (Sigma, St. Louis, MO) and incubated with 10 μg/ml brefeldin A (Sigma) for 4 hours at 37°C in a platform mixer. Red blood cells were then lysed in fluorescence-activated cell sorting (FACS) lysing solution (BD Biosciences, San Jose, CA), the remaining cells were permeabilized in FACS permeabilizing buffer (BD Biosciences), and fluorescence-conjugated antibodies were added for 30 minutes at room temperature in the dark. Cells were then washed and resuspended in 1% wash buffer for flow cytometry. Three-color flow cytometric analysis was performed using a FACSCalibur (BD Biosciences) flow cytometer with CellQuest Pro software (Becton Dickinson). Forward and side scatter were used to gate on the target population (i.e., monocytes). For the monocyte population, the percentage of cytokine-secreting (PE+ and APC+) cells among the CD14+,PerCP+ population was determined by counting ~12,000 CD14+ cells. Resting levels of monocyte expression of proinflammatory cytokines were determined from unstimulated samples that were incubated in the absence of LPS. Net stimulated cytokine-positive events were obtained by subtracting unstimulated percentages from stimulated percentages within constant numbers of monocytes. Results for cytokine-positive monocytes in the LPS-stimulated conditions were expressed as percentages of CD14+ cells.

Statistical analysis. Data were analyzed using SPSS software, version 12.0 (SPSS, Chicago, IL). Group differences in demographic variables were tested using analyses of variance (ANOVAs) or chi-square tests. Stress responses of the 3 groups (healthy controls and RA groups either taking or not taking TNFα antagonists) were tested using repeated-measures ANOVAs for overall differences in a given variable before, during, and/or after the stress task (time effects), overall differences between the 3 groups (group effects), and differential group responses across time (group versus time interactions). Post hoc Bonferroni adjustments were conducted for multiple comparisons. *P* values less than 0.05 were considered significant.

RESULTS

As shown in Table 1, healthy controls and RA patients stratified by use of TNFα antagonists were similar in age, education level, body mass index (BMI), and sex and ethnicity percentages. For plasma IL-6 and CRP levels, there were trends for group differences, and the mean levels in both RA groups were higher than those in the controls ($P < 0.10$). ANOVAs comparing only the 2 RA groups indicated they had similar disease severity, as indicated by DAS28-CRP scores and estimated duration of RA ($P > 0.10$). Approximately 70% of patients in the 2 RA groups took nonbiologic disease-modifying antirheumatic drugs (DMARDs), such as methotrexate, hydroxychloroquine, sulfasalazine, and/or gold, and nearly 40% took NSAIDs. No subject took >10 mg of oral prednisone, although 36% of the RA patients taking TNFα antagonists also took <10 mg/day oral prednisone. Among those RA patients not taking TNFα antagonists, none took oral steroids.

Table 1. Demographic and disease severity characteristics in each group*

	Healthy controls (n = 20)	RA patients: Taking TNFα antagonists (n = 11)	RA patients: Not taking TNFα antagonists (n = 10)	*P*
Age, years	43 ± 12	45 ± 10	47 ± 12	0.69 (F = 0.4)
Education level, years	16 ± 3	15 ± 2	15 ± 2	0.87 (F = 0.2)
BMI, kg/m^2	27 ± 7	29 ± 8	26 ± 6	0.37 (F = 1.0)
No. female/no. male	16/4	10/1	8/2	0.71 (χ^2 = 0.7)
No. white/no. African American	15/5	10/1	8/2	0.56 (χ^2 = 1.1)
Plasma IL-6, pg/ml	1.7 ± 1	5.1 ± 6.4	3.8 ± 5.1	0.10 (F = 2.4)
CRP, mg/liter	1.7 ± 2	3.9 ± 3.8	3.0 ± 2.5	0.08 (F = 3.0)
DAS28-CRP, score	NA	3.4 ± 1.3	3.7 ± 1.1	0.54 (F = 0.4)
Disease duration, years	NA	10 ± 8	12 ± 11	0.57 (F = 0.3)
Current treatment, no.				
DMARDs	NA	9	3	0.5 (χ^2 = 0.4)
NSAIDs	NA	8	4	0.5 (χ^2 = 0.3)
Steroids (<10 mg/day)	NA	4	0	0.03 (χ^2 = 4.5)

* Except where indicated otherwise, values are the mean ± SD. RA = rheumatoid arthritis; TNFα = tumor necrosis factor α; BMI = body mass index; IL-6 = interleukin-6; CRP = C-reactive protein; DAS28-CRP = Disease Activity Score in 28 joints using the CRP level; NA = not applicable; DMARDs = disease-modifying antirheumatic drugs; NSAIDs = nonsteroidal antiinflammatory drugs.

Psychological, cardiovascular, and HPA responses following stress. The stress task increased self-reported psychological stress. As shown in Figure 1, members of all 3 groups reported increased stress after the task ($P < 0.0001$). In addition, overall stress levels differed between the groups ($P < 0.001$), such that members of both RA groups had higher overall stress levels than the controls ($P < 0.05$). There was no group-versus-time interaction.

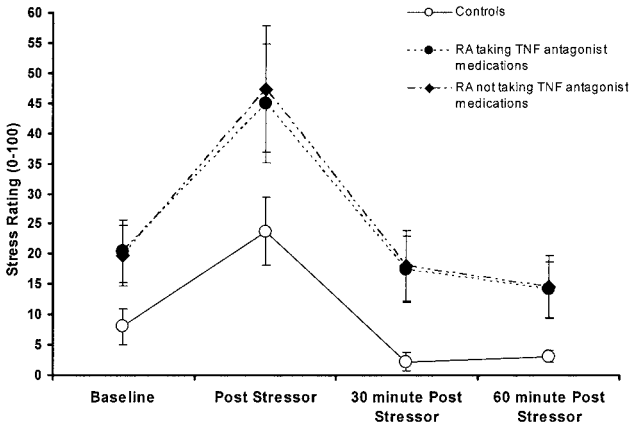

Figure 1. Self-reported psychological stress during the stress task in the 3 groups. The stress task induced significant increases in self-reported psychological stress in all groups (time effect: F[3,117] = 24.5, $P < 0.0001$), and there were significant overall differences between the groups (group effect: F[1,39] = 7.9, $P < 0.001$), such that both rheumatoid arthritis (RA) groups had higher overall levels of stress than did the healthy controls ($P < 0.05$). Values are the mean ± SEM. TNF = tumor necrosis factor.

The stress task also activated cardiovascular measures (Figure 2), with significant changes in systolic blood pressure, diastolic blood pressure, heart rate, and PEP ($P < 0.0001$). However, group members had differential systolic blood pressure responses to stress, as indicated by a significant group-versus-time interaction ($P < 0.002$). As seen in Figure 2A, RA patients taking TNFα antagonists had larger increases in systolic blood pressure during the speaking task than did the controls ($P < 0.05$). For diastolic blood pressure, heart rate, and PEP, there were no overall group differences nor were there any significant group-versus-time interactions.

The stress task also induced similar HPA activation across the groups (Table 2), as evidenced by significant changes in ACTH and cortisol levels over time ($P < 0.001$). There were no overall group differences or group-versus-time interactions for either measure.

Monocyte inflammatory cytokine production following stress. For LPS-stimulated monocyte production of TNFα, members of the groups responded differentially to the stress task ($P < 0.001$) (Figure 3). The groups had similar TNFα production at baseline and immediately after the stress task ($P > 0.05$), but not 30 and 60 minutes later ($P < 0.05$). As shown in Figure 3, RA patients not taking TNFα antagonists had significantly higher TNFα production than did healthy controls at 30 and 60 minutes after the stress task ($P < 0.05$), in contrast to RA patients taking TNFα antagonists, who had levels of TNFα production similar to those of healthy controls at each time point ($P > 0.10$).

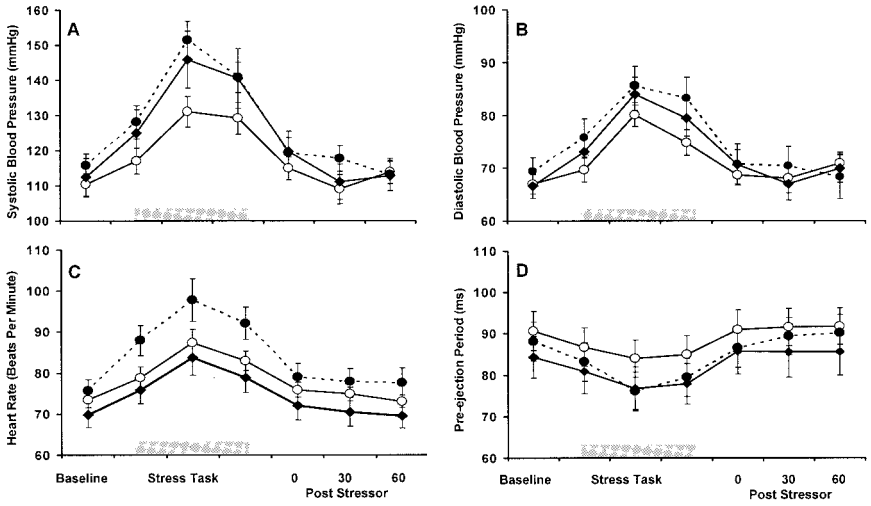

Figure 2. Cardiovascular indices during the stress task in RA patients taking TNFα antagonists (●), RA patients not taking TNFα antagonists (◆), and healthy controls (○). The stress task induced significant cardiovascular activation, as evidenced by significant increases in systolic blood pressure ($F[6,216] = 78.1$, $P < 0.0001$) (**A**), diastolic blood pressure ($F[6,216] = 44.9$, $P < 0.0001$) (**B**), heart rate ($F[6,216] = 49.4$, $P < 0.0001$) (**C**), and a significant decrease in preejection period ($F[6,198] = 46.5$, $P < 0.0001$) (**D**). A significant interaction for systolic blood pressure responses across the groups ($F[6,216] = 2.7$, $P < 0.002$) is shown in **A**. Followup tests indicated that RA patients taking TNFα antagonists had larger increases in systolic blood pressure during the speaking task than did healthy controls ($P < 0.05$). Values are the mean ± SEM. See Figure 1 for definitions.

Because both groups of RA patients had higher self-reported stress levels during the laboratory visit than did healthy controls, we examined whether differences in stress levels across the groups were related to changes in TNFα production. A repeated-measures analysis of TNFα production in all 3 groups was performed, using the stress level at baseline as a covariate. Self-reported stress level was not a significant covariate ($P > 0.10$), and the group-versus-time interaction remained significant ($P < 0.01$). Because steroid use was higher among RA patients taking TNFα antagonists, 2 additional analyses were performed. First, a separate repeated-measures analysis of TNFα production was performed, comparing only the 2 RA groups, with steroid use as a covariate. Results indicated that steroid use did not affect the findings; it was a nonsignificant covariate ($P > 0.10$), and the group-versus-time interaction remained significant ($P < 0.05$). Next, RA patients taking steroids (n = 4) were excluded and a separate repeated-measures analysis was performed. The exclusion of these patients did not change our findings; the group-versus-time interaction remained significant ($P < 0.01$).

Table 2. ACTH and cortisol responses to stress in the overall sample*

	ACTH, pg/ml	Cortisol, ng/ml
Baseline	18.6 ± 9.3	12.6 ± 5.1
Immediately after stress task	20.4 ± 9.7†	13.9 ± 5.7†
After stress task		
30 minutes	16.1 ± 8.5†	11.8 ± 5.3
60 minutes	15.7 ± 8.9†	10.5 ± 5.2†
$F_{time}[3,102]$ (P)	8.9 (<0.001)	9.3 (<0.001)

* Values are the mean ± SD. ACTH = adrenocorticotropic hormone. † $P < 0.05$ versus baseline, by analysis of variance with post hoc Bonferroni adjustment. There were no significant group differences or group-versus-time interactions for either ACTH or cortisol ($P > 0.10$).

For LPS-stimulated monocyte production of IL-6, values tended to decrease over time ($P < 0.05$) (Figure 4). IL-6 production was significantly lower 60 minutes after stress, as compared with immediately after stress ($P < 0.05$); no other time points were different. There was no group effect or group-versus-time interaction for the production of IL-6. For plasma levels of IL-6, stress failed to alter circulating levels of this cytokine over the course of the session ($P > 0.10$).

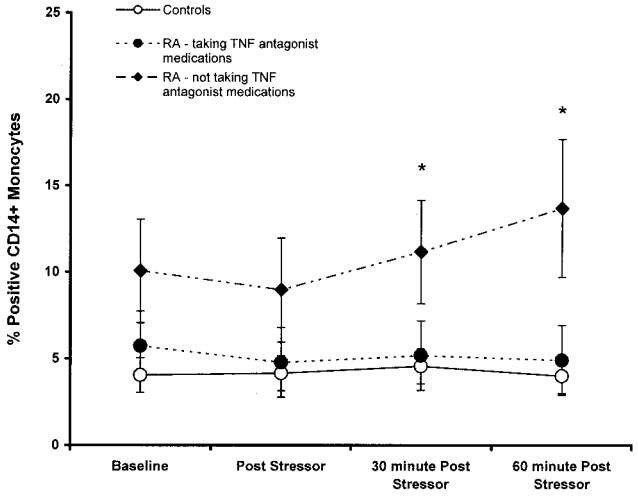

Figure 3. Stimulated production of TNFα by monocytes during the stress task in the 3 groups. The stress task produced a significant group-versus-time interaction for lipopolysaccharide-stimulated monocyte production of TNFα (F[6,84] = 4.0, $P < 0.001$). Followup tests of group differences at each time point revealed that TNFα production was similar among the groups at baseline and immediately after the stress task ($P > 0.05$), but not 30 and 60 minutes after the stress task (F[2,30] = 3.8, $P < 0.05$ and F[2,30] = 5.2, $P < 0.05$, respectively). Pairwise comparisons indicated that RA patients not taking TNF antagonists had higher TNF production than did healthy controls at 30 and 60 minutes after the stress task ($* = P < 0.05$). TNFα production did not differ significantly between RA patients taking TNFα antagonists and healthy controls at any time point. Values are the mean ± SEM. See Figure 1 for definitions.

DISCUSSION

This study is the first to examine the effects of short-term experimental psychological stress on TNFα production in RA patients. Among RA patients not taking TNFα antagonists, stress produced a marked increase in stimulated monocyte production of TNFα as compared with responses in age- and sex-matched healthy controls. In contrast, RA patients taking TNFα antagonists (infliximab, etanercept, or adalimumab) were protected from stress-related increases in TNFα production, with unchanged production throughout the laboratory session similar to that in healthy controls.

TNFα regulates expression of inflammatory cytokines and is an important mediator of bone and cartilage damage in RA (3–5,9). Findings of the current study provide novel information on the effects of psychological stress on TNFα expression in RA and substantially extend the observations of 2 previous stress studies in RA patients. In 1 prior study, RA patients with high disease activity (DAS28 score >4.4) had increased CRP levels 30 minutes after acute psychological stress (7) as compared with those with low DAS28 scores. In the other study, stress failed to induce a differential increase in LPS-stimulated production of IL-6 in RA patients, similar to the negative findings for IL-6 reported here (6). No prior study has examined the impact of TNFα antagonist use on the cellular or in vivo markers of inflammation.

The stress-induced increased TNFα production seen in RA patients not taking TNFα antagonists may reflect altered TNFα regulation at the cellular level. Infliximab, etanercept, and adalimumab work by binding to soluble TNFα, which prevents it from attaching to its receptor, thus rendering the TNFα biologically inactive. There is some evidence that these medications also block the activation of NF-κB, an intracellular transcription factor that initiates expression of genes specific to the production of TNFα and other inflammatory cytokines. IκBα and IκBγ are known inhibitors of NF-κB and are up-regulated in vitro by the TNFα antagonist infliximab (21). Acute psychological stress is known to induce the activation of NF-κB (22,23). Hence, we speculate that TNFα antagonists may block stress-induced increases in TNFα production by altering the NF-κB signaling pathway.

The differential stress-induced increase in TNFα production in the RA groups was not accounted for by differences in clinical variables or treatment with other medications. The 2 RA groups did not differ in any of

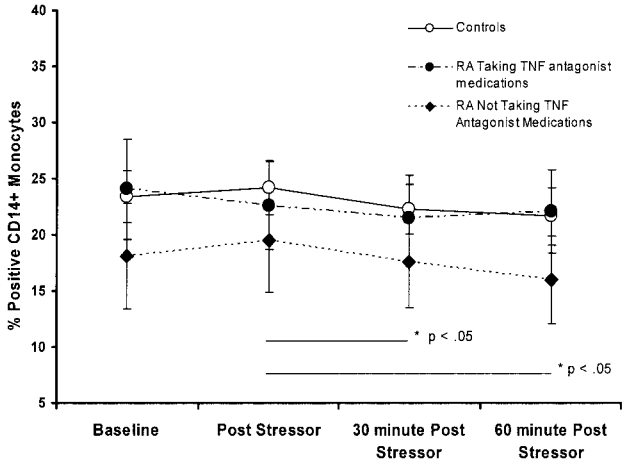

Figure 4. Stimulated production of interleukin-6 (IL-6) by monocytes during the stress task in the 3 groups. The stress task produced a significant time effect for lipopolysaccharide-stimulated monocyte production of IL-6 (F[3,81] = 3.3, $P < 0.05$), with significant differences in the level of IL-6 production for the total sample immediately after the stress task versus 30 and 60 minutes after the stress task. Values are the mean ± SEM. See Figure 1 for other definitions.

the measured demographic variables, including age, BMI, sex, or education. Nor did the RA groups differ in disease-related measures, with similar plasma IL-6 levels, CRP levels, and DAS28 scores. Regarding medications, the 2 groups had similar proportions of DMARD use, and controlling for steroid dose did not affect the results. Group differences in stress-induced TNFα production were not likely related to differences in physiologic stress responses.

Acute stress induces well-delineated increases in perceived stress and in cardiovascular and HPA activity (16), as seen in the current study. Both groups of RA patients reported higher perceived stress than did healthy controls, but this difference was unrelated to cytokine production. The RA groups also had similar responses on cardiovascular measures, including blood pressure, heart rate, and PEP. There is some suggestion that RA patients have blunted (24) or insufficient stress-related cortisol secretion, considering the sustained inflammatory processes involved in RA (25,26); however, this was not found in the current study. It may be that a subgroup of RA patients, namely, those undergoing severe protracted stress, might show altered HPA activity. RA patients and healthy controls had similar levels of ACTH and cortisol. Since cardiovascular and HPA stress responses were similar across the groups, it is unlikely that they can explain the unique increase in TNFα production found in the RA patients not taking TNFα antagonists.

There were a number of limitations in the current study. The increase in TNFα production was highest at 60 minutes after the stress task, the last time point measured in the study. It is not known whether this time point reflects a peak in stress-induced increases in TNFα production or whether later time points might demonstrate even greater increases. The duration of increased TNFα production is also unclear; from the current study, it appears that brief stress lasting $\sim$15 minutes induces an increase in TNFα production 30 and 60 minutes later. Protracted increases in monocyte TNFα production may have relatively greater clinical consequences than changes that are limited in duration. Furthermore, TNFα mediates increases in other inflammatory cytokines, and it is possible that a longer assessment period would reveal increases in expression of other inflammatory cytokines. In particular, assessment of additional cytokines, such as IL-12, IL-17, and interferon-γ, would be important to characterize stress-induced changes in inflammatory cytokines involved in RA.

Although comparable in size to other laboratory stress studies with RA patients (6,7), the sample size in the current study was small. Moreover, the sample was composed of RA patients with mild to moderate disease activity. RA patients in both groups were similar in terms of measures of disease severity, and among those taking TNFα antagonists, disease severity likely reflects the efficaciousness of the medication. Future work should expand these findings to patients who have more severe disease activity.

Last, the study was done in a laboratory setting, and corroborating these findings in stress responses in the RA patient's everyday experiences would be important. Cardiovascular responses following laboratory-based stress are consistent with cardiovascular responses to acute daily stress in the subject's daily life (27). Extension of such work to include measures of inflammatory markers is an important next step. The findings of the current study indicate that RA patients have an altered stress response as compared with healthy controls; whether this is a function of the disease or reflects a preexisting tendency to respond with a heightened inflammatory process is unclear and warrants further study (28).

In conclusion, brief psychological stress, lasting as little as 15 minutes, can trigger increased monocyte production of TNFα in RA patients who are not receiving treatment with TNFα antagonists. Subsequent work examining how psychological stress affects signal transduction of TNFα would help to explain why RA patients may be particularly prone to flares in disease activity following stress. If future studies corroborate this finding, use of TNFα antagonists may be particularly helpful for those RA patients who are vulnerable to the effects of psychological stress.

AUTHOR CONTRIBUTIONS

Dr. Motivala had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Motivala, Irwin.

Acquisition of data. Motivala, Khanna, FitzGerald.

Analysis and interpretation of data. Motivala, Khanna, FitzGerald, Irwin.

Manuscript preparation. Motivala, Khanna, Irwin.

Statistical analysis. Motivala.

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