



ELSEVIER

Enhanced expression of cytokines and chemokines by blood monocytes to in vitro lipopolysaccharide stimulation are associated with hostility and severity of depressive symptoms in healthy women

Edward C. Suarez^{a,*}, James G. Lewis^b, Ranga R. Krishnan^a,
Kenneth H. Young^b

^aDepartment of Psychiatry and Behavioral Sciences, Duke University Medical Center, P.O. Box 3328, Durham, NC 27710, USA

^bDepartment of Pathology, Duke University Medical Center, P.O. Box 3712, Durham, NC 27710, USA

Received 1 July 2003; received in revised form 30 December 2003; accepted 5 January 2004

KEYWORDS

Depressive symptoms;
Hostility; Cytokines;
Chemokines; Blood
monocytes; Women

Summary The current study investigated the relation of hostility and severity of depressive symptoms, separately and jointly, to the capacity of blood monocytes to secrete an array of cytokines when stimulated by bacterial lipopolysaccharide (LPS). Subjects were 44 healthy, non-smoking, premenopausal women (aged 23–49 years) not currently taking oral contraceptives. Data were collected during the follicular phase of the menstrual cycle. The Cook–Medley Hostility (Ho) scale and the Beck Depression Inventory (BDI) were used to assess hostility and severity of depressive symptoms, respectively. Dual-color flow cytometry was used to measure the total expression of interleukin (IL)-1 α , IL-1 β , IL-8, tumor necrosis factor (TNF)- α , monocyte chemotactic protein (MCP)-1 and monocyte inflammatory protein (MIP)-1 α in blood monocytes following 4 h in vitro LPS stimulation of whole blood. In analyses adjusting for age, body mass index (BMI), fasting cholesterol, alcohol use, race and 17 β -estradiol (E₂), higher Ho scores were associated with greater LPS-stimulated expression of IL-1 α ($\beta = 0.033$, $p = 0.02$), IL-8 ($\beta = 0.046$, $p = 0.01$) and IL-1 β ($\beta = 0.024$, $p = 0.06$). Higher BDI scores were associated with greater expression of TNF- α ($\beta = 0.042$, $p = 0.02$) and IL-8 ($\beta = 0.045$, $p = 0.04$). The linear combination of Ho and BDI scores was significantly associated with IL-1 β ($\beta = 0.18$, $p = 0.057$), IL-8 ($\beta = 0.36$, $p = 0.01$), TNF- α ($\beta = 0.25$, $p = 0.03$), and IL-1 α ($\beta = 0.18$, $p < 0.07$). Thus, in healthy women, these psychological risk factors, alone and in combination, induce a proinflammatory phenotype in circulating monocytes characterized by the up-regulation of proinflammatory cytokines, supporting the hypothesis that inflammation

*Corresponding author. Tel.: +1-919-684-2941; fax: +1-919-668-6419.

E-mail address: ecs.jr@duke.edu (E.C. Suarez).

may be a key pathway whereby hostility and depressive symptoms contribute to atherosclerosis and subsequent coronary heart disease (CHD).

© 2004 Elsevier Ltd. All rights reserved.

1. Introduction

Hostility and severity of depressive symptoms have been independently associated with increased risk of coronary heart disease (CHD) in community samples of initially healthy women with no known cardiovascular disease (Ford et al., 1998; Knox et al., 1998; Ferketich et al., 2000; Myrtek, 2001; Wuslin and Singal, 2003). It has also been demonstrated that even subclinical levels of depressive symptoms are associated with future CHD risk (Ferketich et al., 2000; Penninx et al., 2001). For the most part, the relations of hostility and severity of depressive symptoms to CHD appear to be independent of established risk factors, such as age, gender, diabetes, hypertension, and cholesterol (Jiang and Blumenthal, 2003). Although it is not well understood what mechanisms underlie these associations, both behavioral (e.g., increased smoking, decreased physical fitness, poor diet) and biological factors (e.g., excessive sympathetic nervous system and hypothalamic-pituitary activation to stressors) have been implicated (Smith, 1994; Musselman et al., 1998). It is now recognized, however, that CHD is an inflammatory disease characterized by the accumulation of inflammatory cells at the site of atherosclerotic plaque and the up-regulation of proinflammatory cytokines and chemokines (Ross, 1999). One critical cell contributing to CHD is the blood monocyte/macrophage. It has been shown that blood monocytes/macrophages (a) are key cellular components in every phase of the inflammatory process leading to cardiovascular disease (Stary et al., 1994; Ross, 1999; Lessner et al., 2002); (b) predict premature occurrence of coronary events (Olivares et al., 1993); (c) are particularly important in early lesion development (Gerrity, 1981); and (d) are recruited by cytokines (Kim et al., 2000; Litovsky et al., 2003). Studies have shown that circulating inflammatory cytokines and monocyte-associated responses to in vitro stimulation are associated not only with coronary syndromes (Liuzzo et al., 2001) but also with traditional cardiovascular risk factors (Bermudez et al., 2002) and increased risk of CHD (Ridker et al., 1997) in initially healthy women.

Recently our group has presented data showing that, in healthy men, severity of depressive symp-

toms is associated with greater endotoxin-stimulated expressions of monocyte-associated cytokines and chemokines (Suarez et al., 2003) and, in conjunction with hostility, circulating levels of plasma IL-6 (Suarez, 2003a,b). We also have presented data to demonstrate that in men, hostility alone is associated with an enhanced lipopolysaccharide (LPS)-induced expression of tumor necrosis factor (TNF)- α on circulating monocytes (Suarez et al., 2002). Although preliminary, these observations support the hypothesis of an association between proinflammatory cytokines and psychological coronary risk factors of hostility and severity of depressive symptoms in men. To date, parallel studies in women have not been conducted. Given our prior observations in men and the relative importance of proinflammatory cytokines and monocytes in the development of CHD (Bermudez et al., 2002), we hypothesized that hostility and severity of depressive symptoms would be similarly associated with greater monocyte-associated expression of cytokines and chemokines in healthy women. The purpose of the present study, therefore, was to critically test this hypothesis by examining the separate and combined effects of hostility and severity of depressive symptoms on the expression of interleukin (IL)-1 α , IL-1 β , IL-8, TNF- α , monocyte chemotactic protein (MCP)-1 and monocyte inflammatory protein (MIP)-1a following in vitro stimulation of blood monocytes.

2. Methods

2.1. Subjects

Participants were 44 healthy premenopausal women with a mean age of 33.5 years (range: 23–49) who were recruited via advertisements placed in local newspapers. Interested individuals were screened for pre-existing medical and psychological conditions and use of medications that could alter the expression of monocyte markers. None of the participants reported a history or current diagnosis of cardiovascular disease, immune-related conditions, hypertension, substance abuse problems, oral/dental conditions or any other medical/psychiatric conditions. None of

the women were taking oral contraceptives, or any prescription or over-the-counter medications, including low-dose aspirin and/or vitamin supplements. Lastly, individuals who smoked in the last 2 years or had irregular menstrual cycles were excluded. Subjects who met eligibility criteria were scheduled for their laboratory session between 08:00 and 09:00 h following a 12 h overnight fast. Participants were scheduled during the follicular phase (days 5–10) of the menstrual cycle in order to minimize the influence of menstrual cycle phase on expression of monocyte markers (Rogers and Eastell, 2001). Confirmation of follicular phase was done via assessment of 17 β -estradiol (E₂) and progesterone (P) on blood samples collected on the day of the study.

On the day of the laboratory session, all subjects gave written informed consent to participate in the study, approval of which was granted by the Institutional Review Board of Duke University Medical Center. After consent was completed, blood samples were drawn using a 21-gauge butterfly needle. Subjects then completed the psychological scales and a short personal background questionnaire. Participants received monetary compensation for their participation.

2.2. Assessment of hostility and severity of depressive symptoms

Assessment of severity of depressive symptoms and hostility was performed using the 21-item Beck Depression Inventory (BDI) (Beck et al., 1999) and the 50 item Cook–Medley Hostility (Ho) scale (Cook and Medley, 1954), respectively. Subjects also completed a short background questionnaire that included questions on health and health-related behaviors (e.g., exercise, alcohol consumption). It has been suggested that the BDI reflects such dimensions as negative attitude toward self, performance impairment and somatic disturbance (Dozois et al., 1998). The Ho scale, on the other hand, is purported to measure cynical beliefs and mistrust of others (Barefoot and Lipkus, 1994) as well as neurotic and antagonistic hostility (Suarez and Williams, 1990). The BDI and the Ho have been reported to have good psychometric properties including adequate internal validity, good test–retest reliability and construct validity (Cook and Medley, 1954; Barefoot and Lipkus, 1994; Beck et al., 1999). Evidence suggests that BDI scores are positively correlated with clinical ratings of depression (Brown et al., 1995; Beck et al., 1999). Similarly, evidence for convergent validity has been reported for the Ho scale (Smith and Frohm, 1985). It has also been reported that the BDI and Ho scales are significantly

correlated in non-clinical samples (Felsten, 1996; Suarez, 2003a,b). Other studies have shown that depression is related to hostility (Weissman et al., 1971; Schless et al., 1974). For the BDI, participants were instructed to reflect on the week prior to study participation in responding to the questions.

2.3. Blood collection and flow cytometry procedures

Procedures for assessment of total cell-associated cytokines and chemokines have been previously described elsewhere (Schultz, 2003; Suarez et al., 2003). Briefly, following an overnight fast, blood samples were drawn between 08:30 and 09:30 h into two 4 ml tubes containing lithium heparin. Samples were placed on ice for approximately 50 min until transferred to the flow cytometry laboratory. At the flow cytometry laboratory, undiluted whole-blood culture was incubated for 4 h at 37 °C alone or stimulated with LPS (1 μ g/ml). Monensin (1 μ g/ml), an inhibitor of protein transport and secretion (Mollenhauer et al., 1990), was simultaneously added to both cultures to inhibit the secretion of synthesized proteins. After stimulation, leukocytes were fixed with paraformaldehyde and permeabilized with saponin. Erythrocytes were lysed with lysing solution. Samples were then separated into 200 μ l aliquots where cells were incubated with anti-CD14 fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody (mAb). For determining cell-associated expression, cells were incubated with phycoerythrin (PE) conjugated anti-cytokines and -chemokines specific mAb. Controls consisted of samples exposed to equal concentrations of tagged irrelevant antibodies of the same isotype.

Dual-color flow cytometry (FACScan, Beckton Dickinson) was done using CellQuest (ver. 3.1) computer software and gating on CD14-positive cells. Results were expressed as the relative mean fluorescent intensity (MFI) derived from at least 10,000 CD14-positive monocytes. MFI was calculated for total cell-associated IL-1 α , IL-1 β , TNF- α , IL-8, MCP-1 and MIP-1 α . Reproducibility of flow cytometry was ensured by regular measurement of calibrated standards.

Blood samples for lipid assessment were collected in standard chemistry tubes (Serum separator Tubes) and fasting total cholesterol was determined enzymatically (Hitachi 704 Analyzer, Boehringer Mannheim Diagnostics, Indianapolis, ID).

2.4. Analyses

Initial inspection of MFI values for all monocyte markers revealed a skewed distribution, thus logarithmic transformations were performed using the formula $\text{Log}_{10}(X+1)$ (Kirk, 1968). Multivariate regression analysis was conducted separately for Ho and BDI scores entered as continuous variables. To examine the combined effect of Ho and BDI, a principal component analysis was performed and a psychological risk factor (PRF) score was generated. The PRF score is the linear combination of Ho and BDI. The continuous PRF score was used in subsequent analyses examining the combined effect of Ho and BDI on monocyte markers. All multiple linear regression analyses included age, body mass index (BMI), fasting total cholesterol (TC), race and alcohol use as covariates. These covariates were selected on the basis of previous observations suggesting an association between these factors and elevations in proinflammatory cytokines (Elneihoum et al., 1997; Mendall et al., 1997) as well as to adjust for possible confounding with measures of hostility and severity of depressive symptoms. In addition, E_2 was also used as a covariate in all analyses given the reported effect of E_2 on production of cyto-

kines in peripheral blood mononuclear cells (Rogers and Eastell, 2001). Given the expected direction of the hypothesized relationships, a one-tail test was used to determine significance of specific effects.

3. Results

Table 1 presents sample characteristics. Overall, 97% of the participants described their health status as good or excellent in the week preceding their participation. Mean levels of E_2 and progesterone (P) were consistent with the follicular phase of the menstrual cycle. As expected, scores on the Ho and BDI scales were significantly correlated ($r = 0.61$, $p < 0.001$). Using established a priori criteria for the BDI, 25% of the participants scored 10 and above, suggesting mild to moderate intensity of depressive symptoms (Beck, 1967; Beck et al., 1999).

Preliminary analyses examined relation of psychological risk factors to monocyte count. Pearson partial correlations revealed that monocyte count was not associated with either BDI

Table 1 Subject characteristics

| | Mean | SD | Range |
|--------------------------------------|--------------|---------------------------------|------------|
| Age (years) | 33.5 | 8.2 | 23–49 |
| Body mass index (kg/m ²) | 24.5 | 5.1 | 18.8–41.8 |
| Total cholesterol (mg/dl) | 160.4 | 34.8 | 88.0–223.0 |
| 17 β -Estradiol (pg/ml) | 67.1 | 61.3 | 5.5–286.0 |
| Progesterone (ng/ml) | 0.83 | 1.6 | 0.01–11.00 |
| Beck Depression | 5.2 | 6.0 | 0–24 |
| Cook–Medley Hostility | 15.2 | 7.1 | 3–35 |
| Ethnicity (%) | | Self-reported health status (%) | |
| Non-Hispanic White | 68 | Excellent | 47.7 |
| Black | 23 | Good | 50.0 |
| Other | 9 | Fair | 2.3 |
| Exercise regularly (%) | | Alcohol use (%) | |
| Yes | 82 | Never/former | 27.3 |
| No | 18 | Infrequent | 43.2 |
| | | Occasionally | 27.3 |
| | | Regularly | 2.3 |
| Monocyte markers | Median (MFI) | Inter-quartile range | |
| Interleukin-1 α | 12.5 | 3.2–40.2 | |
| Interleukin-1 β | 25.4 | 11.1–73.8 | |
| Tumor necrosis factor- α | 20.8 | 6.7–79.7 | |
| Interleukin-8 | 169.6 | 27.4–439.4 | |
| Monocyte chemotactic protein-1 | 1.2 | 0.1–5.2 | |
| Monocyte inflammatory protein-1a | 31.8 | 14.2–57.1 | |

Due to skewness, mean fluorescent intensity (MFI) for monocyte markers are presented as median and interquartile range.

(partial $r = -0.15$, ns) or Ho (partial $r = -0.11$, ns) scores.

Regression models using Ho score as a continuous variable revealed significant Ho effects for IL-1 α ($\beta = 0.033$, SE = 0.015, $t(29) = 2.17$, $p = 0.02$), IL-1 β ($\beta = 0.024$, SE = 0.015, $t(29) = 1.60$, $p = 0.06$), and IL-8 ($\beta = 0.046$, SE = 0.021, $t(29) = 2.24$, $p = 0.01$). Inspection of β -coefficients indicated that increases in Ho scores were associated with greater expression of these monocyte-associated markers.

Similarly, analyses of BDI score entered as a continuous variable revealed significant BDI-effects for IL-8 ($\beta = 0.045$, SE = 0.025, $t(31) = 1.82$, $p = 0.04$), and TNF- α ($\beta = 0.042$, SE = 0.02, $t(31) = 2.09$, $p = 0.02$). Higher BDI scores were associated with greater expression of IL-8 and TNF- α following LPS stimulation.

Lastly, analyses were performed to examine the combined effect of hostility and severity of depressive symptoms. A principal component analysis yielded a single factor score, PRF (Eigenvalue = 1.61) that accounted for 81% of the variance. As with all factor-analytically derived scores, the PRF score has a mean of 0 and a standard deviation of 1. As with Ho and BDI scores, analysis using partial correlation revealed that PRF was not associated with monocyte count (partial $r = -0.15$, ns). Complete results for regression analyses are presented in Table 2.

Results of regression analyses revealed a significant PRF-effect for IL-1 β ($\beta = 0.18$, SE = 0.11, $t(29) = 1.60$, $p = 0.057$), IL-8 ($\beta = 0.36$, SE = 0.15, $t(29) = 2.34$, $p = 0.011$), TNF- α ($\beta = 0.25$, SE = 0.12, $t(29) = 2.03$, $p = 0.032$) and marginally significant effects for IL-1 α ($\beta = 0.18$, SE = 0.12, $t(29) = 1.56$, $p = 0.070$). As illustrated in Fig. 1a–c, higher PRF scores were associated with greater LPS-stimulated expression of monocyte markers.

4. Discussion

The results of the present study showed that hostility and severity of depressive symptoms, separately and in combination, significantly predict LPS-stimulated expression of monocyte-associated cytokines and chemokines in healthy, premenopausal women. Moreover, with the exception of MCP-1, the pattern of individual cytokine expressions associated with hostility and severity of depressive symptoms in women is the same as that previously observed for men whereby higher BDI scores were associated with LPS-stimulated

expression of IL-1 β , TNF- α , IL-8 and MCP-1 by blood monocytes (Suarez et al., 2003). As with our previous study of healthy men, the current observations were observed in multivariate analyses controlling for various factors such as age, BMI, fasting cholesterol, alcohol use, as well as E₂, all factors known to impact proinflammatory cytokines.¹ In addition to statistical adjustments, exclusionary criteria insured that these observations were not confounded with the presence of established risk factors such as smoking, hypertension and diabetes. Thus, these data support the general hypothesis that proinflammatory mechanisms may be one pathway whereby these empirically supported psychological risk factors lead to an increased risk of CHD.

We previously have demonstrated that scores on the Ho scale significantly and positively predicted LPS-stimulated expression of TNF- α in circulating monocytes from healthy men (Suarez et al., 2002). The current study is the first to our knowledge to indicate that hostility, assessed via the Ho scale, is also associated with greater stimulated expression of monocyte-associated cytokines. Previous studies have reported that the Ho scale predicts incidence of CHD (Haynes et al., 1980) as well as risk of myocardial infarction (MI) in initially healthy women (Barefoot et al., 1995). In postmenopausal women with CHD, hostility has been shown to significantly predict recurrent MI in a 4-year follow-up (Chaput et al., 2002). Although previous studies of healthy women have also indicated that the Ho scale is associated with (a) excessive cardiovascular responses to anger arousal (Suarez et al., 1993), and (b) elevations in fasting lipids (Suarez et al., 1998), both known or suspected of leading to increased risk of CHD, the current findings provide preliminary evidence indicating that inflammatory mechanisms may be one pathway whereby hostility contributes to the development and progression of CHD in women.

¹The rationale for including these variables as covariates was to reduce the possibility of confounding as reported in other studies (e.g., Kop et al., 2002; Miller et al., 2002). However, not all of the covariates included in the regression analyses significantly predicted expressions of monocyte markers. Results of regression analyses showed that fasting TC significantly predicted IL-1 α ($\beta = 0.01$, $p = 0.002$); IL-1 β ($\beta = 0.008$, $p = 0.03$); IL-8 ($\beta = 0.01$, $p = 0.01$); TNF- α ($\beta = 0.009$, $p = 0.02$). 17 β -estradiol predicted IL-8 ($\beta = -0.005$, $p = 0.039$) and race predicted MIP-1a ($F(3, 18) = 3.23$, $p = 0.046$). Although BMI, age and alcohol use failed to predict expression of monocyte markers, these variables have been previously shown to alter the relation of depression to proinflammatory biomarkers (e.g., Kop et al., 2002; Miller et al., 2002), thus it was important to retain these variables in the analyses.

Table 2 β -coefficients (SE) for regression models with psychological risk factor (PRF) score. PRF represents the linear combination of the scores on the Beck Depression Inventory and Cook–Medley Hostility Inventory. All cytokine values were logarithmically transformed

| | IL-1 α | | IL-1 β | | IL-8 | | TNF- α | | MIP1a | | MCP-1 | |
|-------------------|-----------------------|-------|--------------------|-------|----------------------|-------|---------------------|-------|----------------------|-------|----------------------|-------|
| | β | SE | β | SE | β | SE | β | SE | β | SE | β | SE |
| PRF | 0.18 ^{***} | 0.12 | 0.18 ^{**} | 0.11 | 0.36 ^{**} | 0.15 | 0.25 ^{**} | 0.12 | 0.07 | 0.09 | 0.05 | 0.15 |
| Estradiol | -0.002 ^{***} | 0.002 | -0.002 | 0.002 | -0.005 ^{**} | 0.002 | -0.003 | 0.002 | -0.004 | 0.002 | -0.001 | 0.001 |
| Age | 0.002 | 0.010 | 0.000 | 0.010 | -0.002 | 0.001 | 0.000 | 0.011 | -0.009 | 0.013 | 0.002 | 0.002 |
| Body mass index | -0.015 | 0.016 | -0.004 | 0.017 | 0.028 | 0.022 | -0.007 | 0.019 | -0.012 | 0.025 | -0.030 | 0.022 |
| Total cholesterol | 0.010 [*] | 0.002 | 0.008 [*] | 0.003 | 0.010 [*] | 0.004 | 0.009 ^{**} | 0.003 | 0.002 | 0.004 | 0.005 ^{***} | 0.003 |
| Alcohol use | -0.068 | 0.069 | 0.052 | 0.071 | -0.031 | 0.010 | 0.032 | 0.077 | 0.009 | 0.086 | -0.091 | 0.080 |
| Race | 0.022 | 0.084 | -0.028 | 0.087 | -0.192 | 0.117 | -0.081 | 0.094 | -0.141 ^{**} | 0.115 | 0.032 | 0.099 |

^{*} $p < 0.01$.

^{**} $p < 0.05$.

^{***} $p < 0.10$.

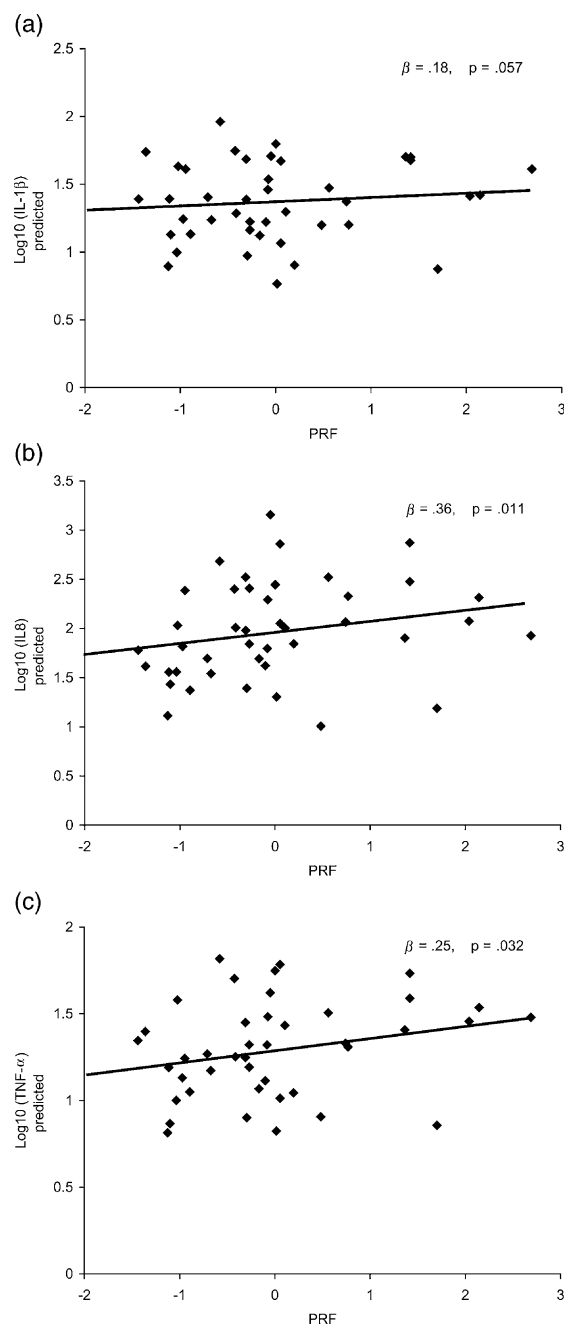


Fig. 1. (a–c) Associations between psychological risk factor (PRF) score and predicted values of logarithmic-transformed mean fluorescent intensity (MFI) for monocyte-associated total expression of interleukin (IL)-1 β , tumor necrosis factor (TNF)- β and IL-8 following 4 h of LPS-stimulation. Log-transformed MFI values are adjusted for age, body mass index, fasting total cholesterol, race, 17 β -estradiol and alcohol use.

Greater LPS-stimulated expressions of monocyte-associated markers were also significantly associated with greater severity of depressive symptoms as indicated by higher BDI scores. Although previous studies have indicated that both severity of depressive symptoms and clinical

depression are associated with higher circulating levels of proinflammatory cytokines, multivariate analyses controlling for traditional risk factors of CHD have led to diminished and non-significant associations (Kop et al., 2002; Miller et al., 2002). In these data, however, the relations of BDI scores to monocyte-associated inflammatory cytokines were significant in multivariate analyses that controlled for various factors. That we excluded individuals with preexisting chronic and acute medical conditions and those women taking prescribed or over-the-counter medications insured that the current results do not reflect potential confounds. Thus, these data suggest that independent of established risk factors of CHD, severity of depressive symptoms is significantly and independently associated with greater expression of monocyte-associated inflammatory markers in healthy women.

Lastly, the linear combination of hostility and severity of depressive symptoms significantly predicted greater expression of monocyte-associated cytokines and chemokines. Not only do these data show an association of the combination of hostility and severity of depressive symptoms with increased expression of proinflammatory cytokines in monocytes, but these results also indicate a quantitative correlation; the more severe the depressive symptoms and greater hostility the greater the production of cytokines (see Fig. 1). Although similar analyses have not been conducted in men, the same linear combination of severity of depressive symptoms and hostility predicts higher levels of plasma IL-6 in healthy, non-smoking men (Suarez, 2003a,b). Taken together, the data from our laboratory underscore the importance of examining the effect of clustering of psychological risk factors on inflammatory biomarkers with the comorbidity of hostility and severity of depressive symptoms as a potentially proatherogenic combination.

There is abundant evidence to indicate that exposure of monocytes and macrophages to LPS induces a reproducible pattern of changes including the up-regulation and secretion of a various cytokines (Kang et al., 1992). In the current study, the same pattern was observed in that all cytokines showed up-regulation following exposure to LPS. However, it is important to note that the enhanced expression of cytokines following LPS significantly varied by level of hostility, severity of depressive symptoms, and their combination. Hostility enhanced expression of IL-1 α , IL-1 β , IL-8, and MIP-1, whereas severity of depressive symptoms was associated with an enhanced expression of TNF- α and IL-8, and the combination of these two factors enhanced the

expression of IL-1 β , IL-8, and TNF- α ; three distinct but yet over-lapping patterns of enhancement. The most likely interpretation of these data is that severity of depressive symptoms and hostility do not simply up-regulate the overall response of monocytes to LPS but, via some unknown mechanism, act on enhancing LPS-stimulated expression of individual cytokines. The novelty of these observations does not allow us to conclude that only certain cytokines are more likely to be affected by psychological risk factors.

The cross-sectional design of the current study also does not allow for any conclusion to be made regarding the directionality of the observed associations. Nevertheless, our observations are in line with the hypothesis proposed by Smith, who suggested that excessive secretion of macrophage products, such as IL-1, causes depression (Smith, 1991). Recent reviews by both Seidel et al. (1999) and Capuron and Dantzer (2003), however, have concluded that while a number of studies report increases in circulating levels of inflammatory cytokines in patients with clinical depression, this has not always been the case. These authors have suggested that the observations of increased circulating levels of cytokines in depressed patients may be due to various confounding factors (e.g., age, BMI, ongoing or recent infections, smoking habits, and prior medications) as well as diagnosis (Haack et al., 1999). Clearly, this is not the case in the current study since all of these factors were either adjusted for in the analyses (e.g., age, BMI) or controlled methodologically via the selection criteria (e.g., no ongoing or recent infection(s), non-smokers, no current or prior medication use including low-dose aspirin, oral contraceptives, vitamin supplements). Thus, while conclusions about causality are not possible, the current data are consistent with the hypothesis of cytokine-induced depressive symptoms independent of various confounding factors.

Although the current findings are similar to our previous observations in healthy men, it is well recognized that premenopausal women are relatively protected from atherosclerosis and CHD (Higgins and Thom, 1993). Nevertheless, one recent study has suggested the importance of modifying risk factors during the pre- and perimenopausal years of a woman's life and that the lack of modification may lead to greater numbers of women requiring more aggressive treatment during their postmenopausal years (Derby et al., 2003). The extent to which inflammation plays a role in future risk of CHD combined with evidence from prospective and case-control studies of the relation of hostility and severity of depressive

symptoms to CHD in women leads to the conclusion that modifications of hostility and reduction of depressive symptoms may subsequently contribute to lower risk of CHD later in life via reduction in inflammation.

In conclusion, these findings show that hostility and severity of depressive symptoms, separately and in combination, predict greater expression of monocyte-associated cytokines and chemokines following *in vitro* stimulation of whole blood by LPS. These associations were significant in multivariate analyses that adjusted for age, BMI, fasting total cholesterol, alcohol use, and race and with methodological criteria insuring that participants were free of ongoing or previous medical conditions associated with inflammation and were also non-smokers. Given these observations, the findings indicate that inflammatory mechanisms may serve as a pathophysiological pathway whereby hostility and severity of depressive symptoms contribute to the atherosclerotic process leading to CHD in women.

Acknowledgements

The authors are grateful to Karen Achanzar and the Comprehensive Cancer Center for Flow Cytometry under the direction of Michael J. Cook, Ph.D. for conducting the flow cytometry studies. We also wish to thank Melanie Tirronen, Sarah S. Rush, and Tara N. Pennington for their efforts in data collection. The authors also wish to acknowledge the initial contributions of Dolph O. Adams, M.D. who was instrumental in initiating these studies. Dr. Adams passed away prior to the collection of data. This work was supported by National Heart, Lung, and Blood Institute grants HL-56105 and HL-67459 (to E.C.S.)

References

- Barefoot, J.C., Lipkus, I.M., 1994. The assessment of anger and hostility. In: Sigman, A.W., Smith, T.W. (Eds.), *Anger, Hostility and the Heart*. Lawrence Erlbaum Associates, Hillsdale, NJ, pp. 43–66.
- Barefoot, J.C., Larsen, S., von der Lieth, L., Schroll, M., 1995. Hostility, incidence of acute myocardial infarction, and mortality in a sample of older Danish men and women. *Am. J. Epidemiol.* 142, 477–484.
- Beck, A.T., 1967. *Depression: Causes and Treatment*. University of Pennsylvania Press, Philadelphia.
- Beck, A.T., Steer, R.A., Garbin, M.G., 1999. Psychometric properties of the Beck Depression Inventory: twenty-five years of evaluation. *Clin. Psychol. Rev.* 89, 757–760.
- Bermudez, E.A., Rifai, N., Buring, J., Manson, J.E., Ridker, P.M., 2002. Interrelationships among circulating interleukin-

- 6, C-reactive protein, and traditional risk factors in women. *Arterioscler. Thromb. Vas. Biol.* 22, 1668–1673.
- Brown, C., Shulberg, H.C., Madonia, M.J., 1995. Assessing depression in primary care practice with the Beck Depression Inventory and the Hamilton Rating Scale for Depression. *Psychol. Assess.* 7, 59–65.
- Capuron, L., Dantzer, R., 2003. Cytokines and depression: the need for a new paradigm. *Brain Behav. Immun.* 17, 5119–5124.
- Chaput, L.A., Adams, S.H., Simon, J.A., Blumenthal, R.S., Vittinghoff, E., Lin, F., Loh, E., Matthews, K.A., 2002. Hostility predicts recurrent events among postmenopausal women with coronary heart disease. *Am. J. Epidemiol.* 156, 1092–1099.
- Cook, W.W., Medley, D.M., 1954. Proposed hostility and pharisaic-virtue scales for the MMPI. *J. Appl. Psych.* 38, 414–418.
- Derby, C.A., Fitzgerald, G., Pasternak, R.C., 2003. Application of Adult Treatment Panel III (ATPIII) guidelines to a multi-ethnic cohort of perimenopausal women: inclusion of age > 55 will produce an important shift in the proportions requiring intervention. In: Paper Presented at the 43rd Annual Conference on Cardiovascular Disease Epidemiology and Prevention, Miami, FL.
- Dozois, D.J., Dobson, K.S., Ahnberg, J.L., 1998. The psychometric evaluation of the Beck Depression Inventory—II. *Psychol. Assess.* 10, 83–89.
- Elneihoum, A.M., Falke, P., Hedblad, B., Lindgärde, F., Ohlsson, K., 1997. Leukocyte activation in atherosclerosis: correlation with risk factors. *Atherosclerosis* 131, 79–84.
- Felsten, G., 1996. Hostility, stress, and symptoms of depression. *Person. Individ. Differ.* 21, 461–467.
- Ferketich, A., Schwartzbaum, J., Frid, D., Moeschberger, M., 2000. Depression as an antecedent to heart disease among women and men in the NHANES I study. *Arch. Intern. Med.* 160, 1261–1268.
- Ford, D.E., Mead, L.A., Chang, P.P., Cooper-Patrick, L., Wang, N.Y., Klag, M.J., 1998. Depression is a risk factor for coronary artery disease in men: the precursors study. *Arch. Intern. Med.* 158, 1422–1426.
- Gerrity, R.G., 1981. The role of the monocyte in atherogenesis. I Transition of blood-borne monocytes into foam cells in fatty lesions. *Am. J. Pathol.* 103, 181–190.
- Haack, M., Hinze-Selch, D., Fenzel, T., Kraus, T., Kuhn, M., Schuld, A., Pollmacher, T., 1999. Plasma levels of cytokines and soluble cytokine receptors in psychiatric patients upon hospital admission: effects of confounding factors and diagnosis. *J. Psychiatry Res.* 33.
- Haynes, S.G., Feinleib, M., Kannel, W.B., 1980. The relationship of psychosocial factors to coronary heart disease in the Framingham Study. III. Eight-year incidence of coronary heart disease. *Am. J. Epidemiol.* 111, 37–58.
- Higgins, M., Thom, T., 1993. Cardiovascular disease in women as a public health problem. In: Packard, B. (Ed.), *Cardiovascular Health and Disease in Women*. LeJapc Communications, Inc, Greenwich, CT, pp. 33–41.
- Jiang, W., Blumenthal, J.A., 2003. Depression and ischemic heart disease: overview of the evidence and treatment implication. *Curr. Psychiatry Rep.* 5, 47–54.
- Kang, Y.H., Lee, C.H., Monroy, R.L., Dwivedi, R.S., Odeyale, C., Newball, H.H., 1992. Uptake, distribution, and fate of bacterial lipopolysaccharides in monocytes and macrophages: an ultrastructural and functional correlation. *Electron Microsc. Rev.* 5, 381–419.
- Kim, C.J., Kho, J.C., Gillote-Taylor, K., et al., 2000. Polymerase chain reaction-based method for quantifying recruitment of monocytes to mouse atherosclerotic lesions in vivo: enhancement by tumor necrosis factor-alpha and interleukin-1 beta. *Arterioscler. Thromb. Vas. Biol.* 20, 1976–1982.
- Kirk, R.E., 1968. *Experimental Design: Procedures for the Behavioral Sciences*. Brooks/Cole Publishing, Belmont, CA.
- Knox, S.S., Siegmund, K.D., Weidner, G., Ellison, C., Adelman, A., Catherine, P., 1998. Hostility, social support, and coronary heart disease in the National Heart, Lung and Blood Institute Family Heart Study. *Am. J. Cardiol.* 82, 1192–1196.
- Kop, W.J., Gottdiener, J.S., Tangen, C.M., Fried, L.P., McBurnie, M.A., Walston, J., Newman, A., Hirsch, C., Tracy, R.P., 2002. Inflammation and coagulation factors in persons > 65 years of age with symptoms of depression but without evidence of myocardial ischemia. *Am. J. Cardiol.* 89, 419–424.
- Lessner, S.M., Prado, H.L., Waller, E.K., Galis, Z.S., 2002. Atherosclerotic lesions grow through recruitment and proliferation of circulating monocytes in a murine model. *Am. J. Pathol.* 160, 2145–2155.
- Litovsky, S., Madjid, M., Zarrabi, A., Casscells, W., Willerson, J.T., Naghavi, M., 2003. Superparamagnetic iron oxide-based method for quantifying recruitment of monocytes to mouse atherosclerotic lesions in vivo: enhancement by tissue necrosis factor- α , interleukin-1 β , and interferon- γ . *Circulation* 107, 1545–1549.
- Liuzzo, G., Angiolillo, D.J., Buffon, A., Rizzello, V., Colizzi, C., Ginnetti, F., Biasucci, L.M., Maseri, A., 2001. Enhanced response of blood monocytes to in vitro lipopolysaccharide-challenge in patients with recurrent unstable angina. *Circulation* 103, 2236–2241.
- Mendall, M.A., Patel, P., Asante, M., Ballam, L., Morris, J., Strachan, D., Camm, A., Northfield, T., 1997. Relation of serum cytokine concentrations to cardiovascular risk factors and coronary heart disease. *Heart* 78, 273–277.
- Miller, G.E., Steller, C.A., Carney, R.M., Freedland, K.E., Banks, W.A., 2002. Clinical depression and inflammatory risk markers for coronary heart disease. *Am. J. Cardiol.* 90, 1279–1283.
- Mollenhauer, H.H., Morre, D.J., Rowe, L.D., 1990. Alteration of intracellular traffic by monensin: mechanism, specificity and relation to toxicity. *Biochim. Biophys. Acta* 1031, 225–246.
- Musselman, D.L., Evans, D.L., Nemeroff, C.B., 1998. The relationship of depression to cardiovascular disease. *Arch. Gen. Psychiatry* 55, 580–592.
- Myrtek, M., 2001. Meta-analyses of prospective studies on coronary heart disease, type A personality, and hostility. *Int. J. Cardiol.* 79, 244–251.
- Olivares, R., Ducimetiere, P., Claude, J.R., 1993. Monocyte count: a risk factor for coronary heart disease? *Am. J. Epidemiol.* 137, 49–53.
- Penninx, B.W., Beekman, A.T., Honig, A., Deeg, D.J., Schoneveld, R.A., van Eijk, J.T., van Tilburg, W., 2001. Depression and cardiac mortality: results from a community-based longitudinal study. *Arch. Gen. Psychiatry* 58, 221–227.
- Ridker, P., Cushman, M., Stampfer, M., Tracy, R.E., Hennekens, C., 1997. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men and women. *N. Engl. J. Med.* 336, 973–979.
- Rogers, A., Eastell, R., 2001. The effect of 17 β -estradiol on production of cytokines in cultures of peripheral blood. *Bone* 29, 30–34.
- Ross, R., 1999. Atherosclerosis—an inflammatory disease. *N. Engl. J. Med.* 340, 115–126.
- Schless, A.P., Mendels, J., Kipperman, A., Cochrane, C., 1974. Depression and hostility. *J. Nerv. Ment. Dis.* 159, 91–100.
- Schultz, C., 2003. Intracytoplasmic detection of proinflammatory cytokines and chemokines in monocytes by flow

- cytometry. In: Korholz, D., Weiss, W. (Eds.), *Cytokines and Colony Stimulating Factors: Methods and Protocols*, 215. Humana Press Inc, Totowa, NJ, pp. 29–39.
- Seidel, A., Rothermundt, M., Rink, L., 1999. Cytokine production in depressed patients. *Adv. Exp. Med. Biol.* 461, 47–57.
- Smith, R.S., 1991. The macrophage theory of depression. *Med. Hypotheses* 35, 298–306.
- Smith, T.W., 1994. Concepts and methods in the study of anger, hostility, and health. In: Siegman, A.W., Smith, T.W. (Eds.), *Anger, Hostility and the Heart*. Lawrence Erlbaum Associates, Hillsdale, NJ, pp. 23–42.
- Smith, T.W., Frohm, K.D., 1985. What's so unhealthy about hostility? Construct validity and psychological correlates of the Cook and Medley Ho scale. *Health Psychol.* 4, 503–520.
- Stary, H.C., Chandler, A.B., Glagov, S., Guyton, J.R., Insull, W.J., Rosenfeld, M.E., Shaffer, S.A., Schwartz, C.J., Wagner, W.D., Wissler, R.W., 1994. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis: a report from the Committee on Vascular Lesions of the Council of Arteriosclerosis, American Heart Association. *Arterioscler. Thromb.* 14, 840–856.
- Suarez, E.C., 2003a. The joint effect of hostility and depressive symptoms on plasma interleukin-6 in apparently healthy men. *Psychosom. Med.* 65, 523–527.
- Suarez, E.C., 2003b. Plasma interleukin-6 is associated with psychological risk factors of atherosclerotic cardiovascular disease: moderation by multivitamin supplementation. *Brain Behav. Immun.* 17, 296–303.
- Suarez, E.C., Williams, R.B., 1990. The relationships between dimensions of hostility and cardiovascular reactivity as a function of task characteristics. *Psychosom. Med.* 52, 558–570.
- Suarez, E.C., Harlan, E.S., Peoples, M.C., Williams, R.B., 1993. Cardiovascular and emotional responses in women: the role of hostility and harassment. *Health Psychol.* 12, 459–468.
- Suarez, E.C., Bates, M.P., Harralson, T.L., 1998. The relation of hostility to lipids and lipoproteins in women: evidence for the role of antagonistic hostility. *Ann. Behav. Med.* 20, 59–63.
- Suarez, E.C., Lewis, J.G., Kuhn, C.M., 2002. The relation of aggression, hostility, and anger to lipopolysaccharide-stimulated tumor necrosis factor (TNF)- α by blood monocytes of normal men. *Brain Behav. Immun.* 16, 675–684.
- Suarez, E.C., Krishnan, R.R., Lewis, J.G., 2003. The relation of severity of depressive symptoms to monocyte-associated proinflammatory cytokines and chemokines in apparently healthy men. *Psychosom. Med.* 65, 362–369.
- Weissman, M., Klerman, G.L., Paykel, E.S., 1971. Clinical evaluation of hostility in depression. *Am. J. Psychiatry* 128, 450–455.
- Wuslin, L.R., Singal, B.M., 2003. Do depressive symptoms increase the risk for the onset of coronary disease? A systematic quantitative review. *Psychosom. Med.* 65, 201–210.