Stress-induced changes in the expression of monocytic β_2-integrins: The impact of arousal of negative affect and adrenergic responses to the Anger Recall Interview

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ABSTRACT

Adhesion of circulating monocytes to the vascular endothelium is one of the earliest steps in the development of atherosclerosis. This leukocyte-to-endothelium interaction is mediated in part by β_2-integrins, a group of cell adhesion molecules that bind to endothelial ligands. Given the significance of this interaction to atherogenesis, we examined the effects of stress, operationalized as the arousal of negative affect (NA) and cardiovascular and catecholamine responses to the Anger Recall Interview (ARI), on the expression of LFA-1 (CD11a), Mac-1 (CD11b) and p150/95 (CD11c) on circulating monocytes (CD14+). Subjects were 173 healthy, nonsmoking men and women (60% men, 40% minorities, aged 18–49 year). Arousal of NA, cardiovascular responses (heart rate [HR], systolic blood pressure [SBP], diastolic blood pressure [DBP]), circulating catecholamines (epinephrine [Epi], norepinephrine [Ne]) and β_2-integrin (CD11/CD18) expression were determined prior to and following the ARI. The principal findings were that the ARI, on average, induced a decrease in monocyte expression of β_2-integrins. However, after adjusting for age, sex, body mass index, exercise status, and baseline level of β_2-integrin expression, those individuals who showed the largest increases in NA, Ne and DBP during the ARI showed an increase in monocyte β_2-integrin expression. Thus, heightened psychological and physiological stress responses induced phenotypic changes in monocytic expression of β_2-integrins that are consistent with the role of monocytes/macrophages in vascular inflammation and increased risk of atherosclerotic cardiovascular disease.

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1. Introduction

Adhesion of circulating monocytes to the vascular endothelium is one of the earliest detectable events in the development of atherosclerosis (Ross, 1999). The firm adherence of circulating monocytes to endothelial cells is mediated by the β_2-integrins, a group of cell surface receptors characterized by an α-chain (CD11a, -b, -c and -d) noncovalently linked to a β-chain (CD18) that is identical for all β_2-integrins (Gamberg et al., 1998). Activation of β_2-integrins is induced by cytokines, chemotactic factors and coagulation factors, as well as by binding to cell surface receptors (Carlos and Harlan, 1994). Heightened expression of β_2-integrins along with their counter-structures on endothelial cells (e.g., ICAM-1 for CD11a and CD11b) promotes transendothelial migration of activated monocytes. This process is then followed by monocytic phagocytosis of intravascular lipids and secretion of proinflam-

matory cytokines, including interleukin (IL)-6 and tumor necrosis factor (TNF)-α (Blake and Ridker, 2001). Proinflammatory cytokine production and increased expression of vascular cell adhesion molecules (CAMs) results in exacerbated inflammation at the site of the early lesion and eventual progression to atherosclerotic plaque (Best and Gersh, 2001; van der Meer et al., 2002).

Negative affective states and traits such as anger and hostility have been associated with an increased risk of atherosclerotic cardiovascular disease (ACVD) (Rozanski et al., 2005). At this time, however, the underlying mechanisms that account for these associations are not well understood. We and others have posited that the relation of state and trait measures of psychological distress to ACVD may be due, in part, to inflammation (Black, 2006; Kop et al., 2003; Suarez, 2003; Suarez et al., 2004b, 2006) and particularly the effects of stress on monocyte function. Although a number of studies have shown that psychological distress is associated with elevations in inflammatory biomarkers such as IL-6 and C-reactive protein (CRP) (e.g., Kop et al., 2008; Suarez, 2003; Suarez et al., 2002), there is a paucity of evidence for the relation of psycholog-

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ical distress to changes in the expression of CAMs by circulating monocytes[1]. Of those few studies that have examined these associations the results have been equivocal. For example, one study showed that monocytic surface expression of CD11a decreased following a speech stressor (Mills et al., 2003). In contrast, a different study using the same stressor reported no changes in monocytic expression of CD11a (Goebel and Mills, 2000). Both of those studies were limited in a number of important ways, including (a) relatively small sample size, (b) assessment of only one or two β_2-integrins on monocytes, and (c) analysis of mean effects for the stress task without consideration of individual differences in psychological and/or physiological stress reactivity. The current study, therefore, aimed to clarify the effect of acute psychological stress on monocytic β_2-integrin expression by examining individual differences in stress-induced arousal of negative affect (NA), adrenergic and cardiovascular (CV) reactivity as predictors of changes in monocyte cell surface expression of three β_2-integrins (CD11a, CD11b and CD11c) in a large sample of apparently healthy adults.

2. Methods

2.1. Participants

One-hundred and seventy-three healthy adult men and women were recruited from the local community. Demographic characteristics of the sample are provided in Table 1. A detailed description of recruitment and subject entry criteria have been presented elsewhere (Suarez et al., 2004b). Briefly, subjects were recruited via advertisements placed in local newspapers and fliers distributed throughout the community. Inclusion criteria included, age 18–50 years, nonsmoker defined as less than six cigarettes/lifetime, and a negative history or current diagnosis of chronic medical conditions known to influence inflammatory biomarkers. These conditions included asthma, allergies, arthritis, diabetes, cancer, hypertension and other cardiovascular conditions as well as psychiatric conditions. Women were required not to have used oral contraceptives 6 months prior to participation.

Two weeks prior to their laboratory sessions, subjects were required to be free of acute infections and recent injuries and to have not undergone medical/dental procedures. Subjects were also required not to use prescription medications and over-the-counter preparations, including low-dose aspirin, for 2-weeks prior to the study. Informed consent was obtained prior to the study. Subjects received $70 for their participation. The Institutional Review Board of Duke University Medical Center approved this protocol.

Laboratory sessions for female subjects who were premenopausal were scheduled during the follicular phase (days 5–10) of their menses, a time when ovarian hormones are at their nadir. Confirmation of menstrual cycle phase was determined from blood samples collected on the day of the laboratory study. Samples were assayed for levels of estradiol (E_2) and progesterone (P).

2.2. Experimental protocol

Given our interest in biobehavioral mechanisms of ACVD and the role of NA, the protocol was designed to generate data that would allow us to examine the relationship between arousal of NA and molecular mechanisms implicated in atherosclerosis. Based on the well-documented relationship between negative affectivity and cardiovascular disease, we employed the Anger Recall Interview (ARI) as the laboratory stressor. A detailed account of

Table 1
Distribution, means (SD) and ranges for demographics of study sample.

	Mean (SD)	Range
Characteristics of study participants		
Age (yr)	26.7(7.7)	18–49
BMI (kg/m^2)	24.2(1.9)	16.9–41.8
	N	**%**
Gender		
Male	104	60
Female	69	40
Race		
White	104	60
Black	40	23
Other	29	17
Exercise regularly		
Yes	142	82
No	31	18
Positive family history		
CHD	22	13
Hypertension	29	17
Educational status		
Less than or HS	5	3
Some college or more	168	97

the experimental protocol has been published previously (Suarez et al., 2004b).

Briefly, subjects reported to the laboratory between 8:00 and 9:00 am after an overnight fast. Upon arrival, subjects were seated in a reclined position and a 19-gauge butterfly needle was inserted in the antecubital vein of the right arm and a blood pressure cuff was placed on the left arm. After a 30 min rest period, baseline physiological measures were taken during a 15 min period and this was followed by assessment of negative affect. The 15 min baseline period was followed by a 5 min reading task, a 5 min recovery period, a 5 min ARI, and a 15 min second recovery period. The reading task was used to adjust for any cardiovascular increases associated with vocalization. For the reading task, subjects were asked to read aloud a story about the ocean. To insure minimal emotional arousal, subjects were told that they would not be evaluated for either reading style or comprehension of text. Results of these analyses have been published elsewhere (Suarez et al., 2004b).

The instructions for the ARI directed subjects to verbally describe a personal event that occurred within the past 3 months and that elicited feelings of anger when it occurred and still aroused feelings of anger upon recall. Following a 1 min period of preparation, subjects verbally described the event for approximately 4 min. To assist the subjects in the recall process, the experimenter used prompts such as "How did that make you feel when it happened?"

As previously noted, we and others have shown that the ARI evokes significant arousal of NA (e.g., Boltwood et al., 1993; Gottdiener et al., 2003; Suarez et al., 2004b) as well as robust adrenergic reactivity not only in healthy controls (Suarez et al., 2004b), but also in clinical samples of patients with coronary heart disease (Kop et al., 2008) and those with post-traumatic stress disorder where responses to the ARI indicated reduced parasympathetic system functioning (Hughes et al., 2007). Recent studies have also shown that the ARI can evoke increases in proinflammatory biomarkers, such as CRP, in cardiac patients (Kop et al., 2008) and stimulated production of inflammatory cytokines in healthy controls (Suarez et al., 2006).

The 15-min recovery period following the ARI allowed for any changes in cell surface markers to manifest since studies have suggested that immune changes may occur 15–30 min after the completion of the task (Segerstrom and Miller, 2004).

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[1] The current study focused solely on monocytes and changes in β_2-integrins. Other studies have examined β_2-integrin expression on circulating lymphocytes. As described in the introduction, our focus on monocytes stems from the integral role in atherosclerotic cardiovascular disease.