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Acute stress evokes selective mobilization of T cells that differ in chemokine receptor expression: a potential pathway linking immunologic reactivity to cardiovascular disease

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Abstract

T lymphocytes and monocytes/macrophages are the most abundant cells found in the atherosclerotic plaque. These cells can migrate towards the activated endothelium through the local release of chemotactic cytokines, or chemokines. Given the important role of leukocyte migration in atherosclerosis and the role of stress in mediating leukocyte trafficking, the present study examined the effects of an acute stressor on the redistribution of T cells (CD3+) and monocytes that express the chemokine receptors CCR5, CCR6, CXCR1, CXCR2, CXCR3, and CXCR4. Forty-four undergraduate students underwent a public speaking task. The acute stressor induced sympathetic cardiac activation, parasympathetic cardiac withdrawal, lymphocytosis, and monocytosis (all $p < .001$). Although the total number of T lymphocytes did not change, there was a selective increase in the number of circulating T cells expressing CXCR2, CXCR3, and CCR5. The ligands of these receptors are chemokines known to be secreted by activated endothelial cells. Analyses of individual differences in stress-induced responses demonstrated a positive relationship between sympathetic cardiac reactivity and mobilization of the various T cell subsets ($.35 < r < .56$; $p < .05$). For the monocytes, all sub-populations increased in parallel with total monocyte numbers, with no relation to changes in sympathetic cardiac drive. These results indicate that acute stress induces a mobilization of T cells that are primed to respond to inflamed endothelium. Acute stressors may thus promote the recruitment of circulating immune cells into the sub-endothelia, and therefore accelerate atherosclerotic plaque formation and potentially contribute to the complications that follow acute stressful events. This mechanism may help explain the link between stress, reactivity, and cardiovascular disease.

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1. Introduction

Many lines of evidence, ranging from pathologic analyses to epidemiological studies, show that atherosclerosis is intrinsically an inflammatory disease (Libby, Ridker, & Maseri, 2002; Ross, 1999). The initiation of inflammatory reactions is a complex process involving the coordinated expression of cellular adhesion molecules and chemotactic cytokines (chemokines), which recruit blood-derived leukocytes to the site of inflammation. The recruitment of leukocytes by chemokines into the sub-endothelium of the vascular wall is a major aspect of atherogenesis. T lymphocytes are among the first cells to infiltrate the sub-endothelium (Libby et al., 2002; Ross, 1999; Song, Leung, & Schindler, 2001), and remain a major local cell population throughout the atherosclerotic process. These T cells subsequently secrete cytokines (e.g., interferon- γ , TNF- α , and interleukin-2), which further promote the inflammatory atherosclerotic response. Monocytes are another critical constituent of the atherosclerotic response. Once resident in the vessel wall, monocytes develop into macrophages as they take up oxidized low-density lipoprotein and differentiate into so-called foam cells. Macrophages and lipid-laden foam cells are implicated as prime culprits in the events that ultimately complicate atherosclerosis (Libby et al., 2002; Ross, 1999).

It is likely that the endothelium itself initiates this process of leukocyte recruitment (Libby et al., 2002; Reape & Groot, 1999; Shin, Szuba, & Rockson, 2002). Endothelial cells can secrete numerous chemokines upon activation by molecules derived from the circulation and adjacent cells (e.g., Burke-Gaffney, Brooks, & Bogle, 2002; Kotani, Hori, Matsumura, & Uchiyama, 2002; Mach et al., 1999; Qi & Kreutzer, 1995; Seeger et al., 2002). In fact, virtually all cardiovascular risk factors (e.g., increased LDL levels, hypertension, diabetes, obesity, and infection) are capable of promoting an inflammatory response in endothelial cells with the concomitant secretion of inflammatory mediators (Libby et al., 2002). Chemokines secreted by endothelial cells include Growth Regulated Oncogene (GRO, which has an α , β , and γ sub-type), Epithelial Neutrophil Activating peptide-78 (ENA-78), Neutrophil Activating Protein-2 (NAP-2), and Interleukin 8 (IL-8). These chemokines are all ligands for the pleiotropic chemokine receptor CXCR2, whereas IL-8 can also stimulate the chemokine receptor CXCR1. Other examples of chemokines secreted by endothelial cells are Interferon- γ Inducible Protein-10 (IP-10), which binds to the chemokine receptor CXCR3, and Regulated on Activation Normal T cell Expressed and Secreted (RANTES), which is a ligand for several chemokine receptors including CCR5 (Burke-Gaffney et al., 2002; Oppenheim, Zachariae, & Goetzl, 2000; Wang, Su, Gong, & Oppenheim, 1998).

Acute psychological stressors are known to modulate this process of leukocyte trafficking and to enhance subsequent cellular immune responses in the local tissues (Dhabhar & McEwen, 1997, 1999; Dhabhar, Miller, Stein, McEwen, & Spencer, 1994; Sanders & Straub, 2002). If, as the existing evidence suggests, migratory responses of leukocytes are crucial in the development of atherosclerotic lesions, then acute stress may influence atherosclerotic plaque formation in part through its effects on leukocyte migration and recruitment.

The magnitude of cardiovascular system responses to acute stressors (“cardiovascular reactivity”) is considered a potential risk factor for cardiovascular disease progression and its acute clinical manifestations (Kop, 1999; Krantz, Kop, Santiago, & Gottdiener, 1996; Rozanski, Blumenthal, & Kaplan, 1999; Sheps et al., 2002). Likewise, immune reactivity (the response of immune parameters during acute stress) has been proposed as a potential predictor for vulnerability to immune mediated disease (Cacioppo et al., 1998; Cohen et al., 2002; Sanders & Straub, 2002). Cardiovascular and immune reactivity are correlated phenomena that are both determined by sympathetic nervous system activation (Cacioppo et al., 1995; Sgoutas-Emch et al., 1994; Uchino, Cacioppo, Malarkey, & Glaser, 1995). Thus, it is possible that the observed link between sympathetic cardiac reactivity and cardiovascular disease manifestations is mediated in part through immunological pathways. The present study examined the effects of an acute stressor on the redistribution of T cells (CD3+) and monocytes that express the chemokine receptors CCR5, CCR6, CXCR1, CXCR2, CXCR3, and CXCR4. The findings suggest that immune reactivity, or at least some aspects of this phenomenon, may also be relevant to the development of cardiovascular disease.

2. Methods

2.1. Participants

Forty-four university undergraduates (mean age 20, range 18–27 years, 22 male) volunteered to participate in this study as part of a longitudinal study on psychosocial factors and wound healing. Participants gave written informed consent and received a monetary compensation for their participation. Participants were ineligible if they were using medication, or reported health problems indicative of cardiovascular, inflammatory, or infectious disease.

2.2. Procedures

In preparation for the study, participants were instructed not to engage in strenuous physical exercise, and to refrain from using alcohol or non-prescription

drugs 24 h before the experimental sessions. In addition, participants were instructed to abstain from smoking and caffeine the day of the experiment, to eat breakfast before 10 a.m. and stay null per os (except for water) from 10 a.m. onwards. Women were scheduled in the first week after their menses. Upon arriving at the General Clinical Research Center at 12:00 p.m.; (a) informed consent was obtained; (b) a 19-G indwelling catheter was placed into the antecubital vein of the non-dominant arm; (c) participants were served a standardized lunch (300 kcal, containing 10 g of protein, 12 g of carbohydrate, and 16 g of fat) and water ad libitum; and, (d) electrodes for electrocardiography (ECG) and impedance cardiography (ICG) were attached. Subsequently, while seated in supine position, participants filled out questionnaires and engaged in leisure reading. At 1:30 p.m., a baseline blood sample was obtained and the procedure for the laboratory stressor was initiated.

2.3. Public speaking task

The stress task consisted of two back-to-back speeches, each with 2 min of preparation and 4 min of speech delivery. To enhance social stress, the speeches were videotaped and attended by a small audience consisting of a female nurse, a female research assistant, and a male psychologist. For the first speech, the participant had to defend him/herself after being falsely accused of shoplifting (Saab, Matthews, Stoney, & McDonald, 1989), and for the second speech the participant gave a presentation about his or her best and worst personal characteristics (van Eck, Nicolson, Berkhof, & Sulong, 1996). In order to standardize timing and instructions for the task, the instructions were presented on a video screen. Including instructions, the task took 15 min. A second blood sample was obtained during second minute of the second presentation (13 min after initiation of the task). Cardiac activity was recorded continuously.

2.4. Cardiovascular assessment

Assessment of cardiovascular responses focused on cardiac sympathetic and vagal control (Berntson, Cacioppo, & Quigley, 1993). Indices of sympathetic and parasympathetic drive were obtained by analysis of ECG and thoracic impedance (ICG) signals (Berntson et al., 1997; Sherwood et al., 1990). The thoracic impedance and ECG signals were recorded from six Ag/AgCl spot-electrodes (AMI type 1650-005, Medtronic) using the Vrije Universiteit Ambulatory Monitoring System (VU-AMS) device. Reliability and validity of the VU-AMS device have been reported elsewhere (de Geus, Willemsen, Klaver, & van Doornen, 1995; de Geus & van Doornen, 1996; Willemsen, De Geus, Klaver, Van Doornen, & Carroll, 1996). The ECG and ICG complexes were ensemble averaged with reference to the

ECG R-wave across 1-min periods. From these 1-min ensembles, average levels were computed for heart rate (HR) and preejection period (PEP). These minute-by-minute means were averaged over a 6-min pretask baseline and over each 6-min stressor.

Interbeat intervals (IBI) were checked and edited for artifacts by a detection algorithm developed by Berntson, Quigley, Jang, and Boysen, 1990. Respiratory sinus arrhythmia (RSA) was derived by the method of Porges (Porges & Bohrer, 1990), using the Mxedit program (Delta Biometrics, Bethesda MD). This program converts the heart period series into a time series, applies a moving polynomial filter (polynomial = 3, coefficients = 21) to remove slow non-stationarities in the data, applies a band-pass filter (.12–.40 Hz) to the residual series, and then derives the natural log of the band variance. This corresponds to the statistical variance of the time sampled heart period data within the respiratory frequency band.

Changes in PEP were used to index changes in cardiac sympathetic drive, whereas RSA was used to index changes in cardiac vagal tone.

2.5. Flow cytometry

Whole blood was collected into sodium heparin tubes, and maintained at room temperature. Samples were prepared within 2 h after collection. White blood cell counts were obtained on a hematology analyzer (F800, Sysmex, McGraw Park, IL). Specific leukocyte sub-types were identified by immunofluorescent antibody staining using flow cytometry (FACSCalibur, Becton–Dickinson, San Jose, CA). Whole blood was stained using phycoerythrin (PE) and FITC conjugated monoclonal antibodies for chemokine receptors, and cychrome (CyChr) for CD3 (Pharmingen, San Diego, CA). Briefly, cell suspensions were incubated with antibody for 20 min at room temperature, lysed with FACS Brand Lysing Solution (Becton–Dickinson, San Jose, CA), which results in a simultaneous lysis of red blood cells and partial fixation of leukocytes, washed with PBS, and read on the FACSCalibur with 3000–5000 events being acquired from each preparation. Matched antibody isotype controls were used to set negative staining criteria. Data were analyzed using Cell Quest-pro software (Becton–Dickinson, San Jose, CA).

2.6. Data analysis

Repeated measures ANOVA/ANCOVA was conducted to examine the effects of the laboratory stressor on mood, cardiovascular parameters, and cellular distribution. The ECG recording of one subject could not be completed due to a technical problem. For two subjects, flow cytometry data was incomplete due to incomplete lysis of a blood sample. Degrees of freedom were adjusted accordingly.

3. Results

3.1. Psychological and cardiovascular reactions

Analysis of POMS subscales indicated that the speech tasks were perceived as stressful, as evidenced by increases in tension-anxiety ($M_{\text{baseline}} 3.1$ (SEM 0.5), $M_{\text{task}} 9.3$ (SEM 0.8), $F_{(43)} = 21.34, p < .001$) and anger-hostility ($M_{\text{baseline}} 0.18$ (SEM 0.3), $M_{\text{task}} 2.3$ (SEM 0.6), $F_{(43)} = 10.73, p < .01$). Replicating prior research, analyses confirmed that the acute psychosocial stressor elevated HR, increased cardiac sympathetic activation, and produced vagal withdrawal (see Table 1). Because gender and body composition (i.e., BMI) have been reported to moderate the autonomic responses to acute stressors (as well as being independent predictors of cardiovascular disease occurrence), we included these potential modifiers in our statistical model. As shown in Table 1, controlling for these variables yielded comparable results. Further analyses did neither reveal modifying effects of age or smoking on cardiac responses.

3.2. Cellular responses

Data and statistical analyses for the lymphocytes are presented in Table 2. The acute stressor induced significant lymphocytosis, whereas the total number of circulating T lymphocytes did not increase. Further analysis of T cell sub-population on basis of chemokine receptor expression, revealed significant increases in the

numbers of CD3+CXCR2+, CD3+CXCR3+, and CD3+CCR5+ lymphocytes (see Table 2). Analyses of these sub-populations expressed as% of total T cell numbers yielded a similar pattern of results, showing a selective increase of CXCR2+, CXCR3+, and CCR5+ T-cells within the total circulating T-cell pool (all $F_{(43)} > 6.5, p < .01$). This analysis further revealed a small decrease in CCR6+ T-cells relative to the total number of T cells ($F_{(43)} = 6.18, p < .05$).

Data and statistical analyses for monocytes are presented in Table 3. The speaking stressor increased total monocyte numbers. A general increase was also seen for the various monocyte sub-populations, which paralleled the increase in total monocyte numbers (see Table 3). That all monocyte subsets (as defined by chemokine receptor expression) changed to the same degree was further corroborated by non-significant changes in monocyte subsets expressed as percentage of total monocyte number (data not shown).

We again performed analyses controlling for possible moderating effects of gender and BMI. As shown in Tables 2 and 3, these analyses yielded a similar pattern of results. Subsequent exploratory analyses neither indicated moderating effects of smoking status or age.

3.3. Associations between cellular and cardiac autonomic responses

To confirm the assumption that cardiac sympathetic reactivity and immune reactivity are related phenomena,

Table 1

Mean values (with SEM in parentheses) and results of statistical analyses (repeated measures ANOVA/ANCOVA) for cardiac autonomic measures during baseline and speech tasks

	Baseline	Stressor	$F_{(42)}$	p	Controlling for gender and BMI	
					$F_{(39)}$	p
Heart rate (bpm)	70.7 (1.6)	88.4 (2.2)	88.81	<.001	81.26	<.001
PEP (ms)	102.2 (2.2)	91.5 (2.3)	16.91	<.001	13.25	<.001
RSA (ms)	6.98 (0.12)	6.30 (0.15)	18.77	<.001	17.78	<.001

Table 2

Mean values (with SEM in parentheses) and results of statistical analyses (repeated measures ANOVA/ANCOVA) for lymphocyte cell numbers during baseline and speech tasks

	Baseline	Stressor	$F_{(43)}$	p	Controlling for gender and BMI	
					$F_{(40)}$	p
Total lymphocytes (cells/mm ³)	1757 (72)	2076 (73)	22.99	<.001	19.75	<.001
Total CD3+ (cells/mm ³)	1145 (59)	1208 (51)	1.80	ns	0.93	ns
CD3+ CCR5+ (cells/mm ³)	288 (25)	324 (26)	6.95	<.01	4.65	<.05
CD3+ CCR6+ (cells/mm ³)	463 (30)	452 (27)	1.47	ns	2.41	ns
CD3+ CXCR1+ (cells/mm ³)	421 (49)	439 (49)	0.34	ns	0.10	ns
CD3+ CXCR2+ (cells/mm ³)	243 (26)	301 (28)	12.86	<.001	11.67	<.01
CD3+ CXCR3+ (cells/mm ³)	699 (37)	777 (39)	12.13	<.001	10.52	<.01
CD3+ CXCR4+ (cells/mm ³)	1092 (50)	1137 (49)	2.08	ns	1.12	ns

Table 3

Mean values (with SEM in parentheses) and results of statistical analyses (repeated measures ANOVA/ANCOVA) for monocyte cell numbers during baseline and speech tasks

	Baseline	Stressor	$F_{(43)}$	p	Controlling for Gender and BMI	
					$F_{(40)}$	p
Total monocytes (cells/mm ³)	272 (14)	353 (18)	37.60	<.001	34.00	<.001
CCR5+ (cells/mm ³)	35 (3)	47 (5)	14.68	<.001	11.47	<.01
CCR6+ (cells/mm ³)	94 (8)	113 (9)	7.91	<.01	5.59	<.05
CXCR1+ (cells/mm ³)	247 (13)	318 (17)	35.98	<.001	31.96	<.001
CXCR2+ (cells/mm ³)	203 (16)	264 (20)	25.22	<.001	24.16	<.001
CXCR3+ (cells/mm ³)	22 (2)	28 (3)	8.57	<.01	9.67	<.01
CXCR4+ (cells/mm ³)	226 (13)	284 (16)	26.03	<.001	22.75	<.001

we compared cellular mobilization responses in subjects that exhibited a high versus a low cardiac sympathetic reactivity. The cardiac PEP was utilized as a measure of cardiac sympathetic drive. Reactivity was calculated as $(PEP_{\text{task 1}} + PEP_{\text{task 2}})/2 - PEP_{\text{baseline}}$, and grouping into high and low PEP reactors was based on a median split of these reactivity scores. The average Δ s for the high PEP reactors was -17.2 (SEM 1.8), and -1.5 (SEM 0.6) for the low PEP reactors ($t_{(41)}8.29; p < .001$). High and low PEP reactors also differed in HR reactivity ($t_{(41)}2.47; p < .05; \Delta$ bpm 21.9 (SEM 2.9) vs. 13.3 (SEM 1.8)), whereas the two groups did not differ in vagal responses ($t_{(41)}0.99; ns$).

Ideographic analyses showed a general increase in circulating T cell subsets for high PEP reactors, whereas

low reactors exhibited no change or a decrease (see Fig. 1). Independent t tests yielded significant differences for CD3+CCR5+ ($t_{(40)} = 4.62, p < .001$), CD3+CCR6+ ($t_{(40)} = 3.62, p < .005$), CD3+CXCR1+ ($t_{(39)} = 2.77, p < .05$), CD3+CXCR2+ ($t_{(40)} = 3.72, p < .005$), CD3+CXCR3+ ($t_{(40)} = 3.44, p < .005$), and CD3+CXCR4+ ($t_{(39)} = 3.47, p < .005$) (see Fig. 1). Inspection of the scatter plots and computation of bivariate correlations indicated that the associations between sympathetic cardiac drive and cellular mobilization showed a near linear relationship (see Table 4 and Fig. 2).

Previous studies have reported that individual differences in HR reactivity, instead of PEP, may predict immune reactions e.g. (Benschop et al., 1998; Larson, Ader, & Moynihan, 2001; Sgoutas-Emch et al., 1994),

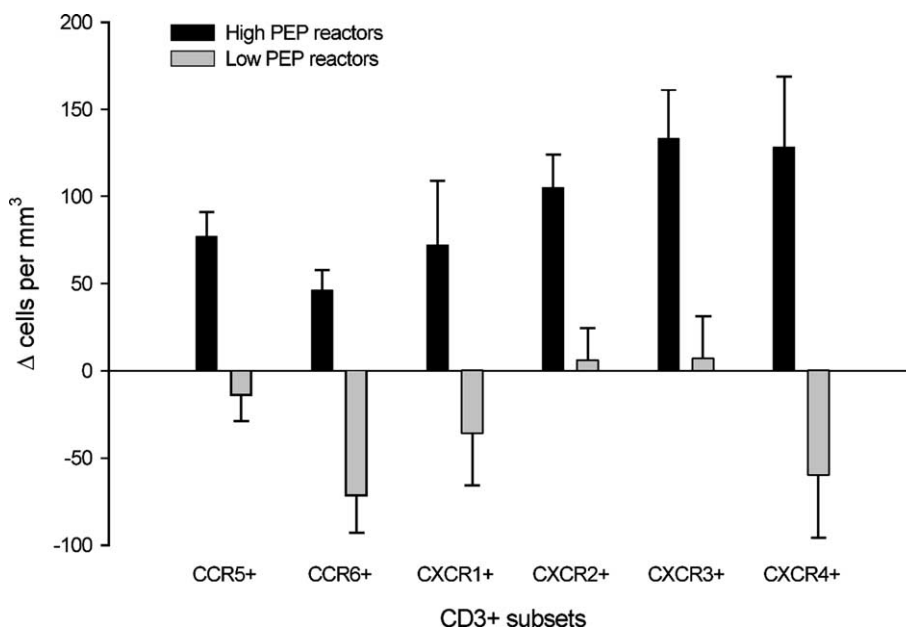


Fig. 1. Change in T cell sub-set numbers for subjects that exhibited a high versus a low cardiac sympathetic response (indexed as changes in PEP) during the speech stressors. Bars indicate mean, lines indicate standard error of mean.

Table 4

Pearson correlation coefficients and Spearman's rank-order correlation coefficients (between brackets) of the association between PEP reactivity and changes in T cell numbers

	CD3+CXC1+	CD3+CXC2+	CD3+CXCR3+	CD3+CXCR4+	CD3+CCR5+	CD3+CCR6+
PEP reactivity (Δ ms)	-.32 (-.35)	-.40 (-.43)	-.37 (-.41)	-.43 (-.42)	-.50 (-.56)	-.35 (-.40)

For all analyses $p < .02$.

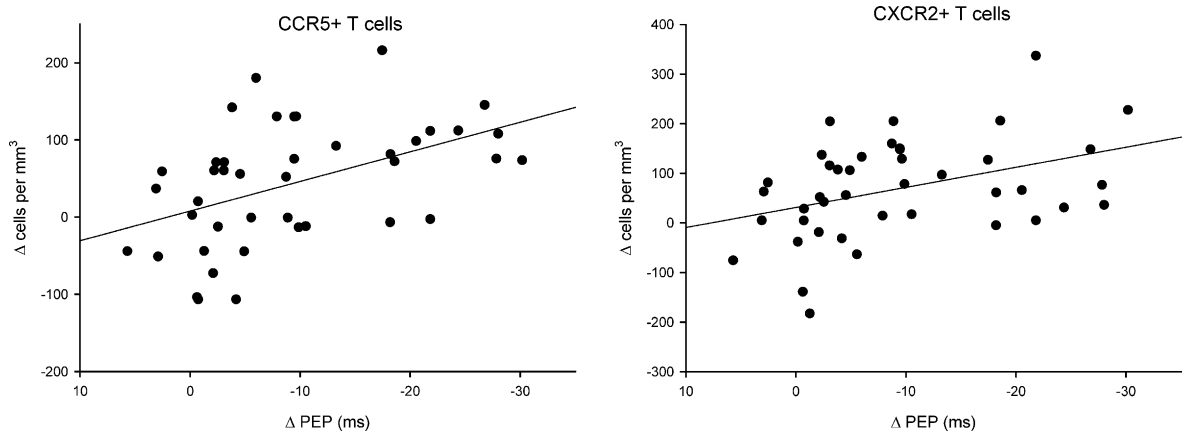


Fig. 2. Scatter plots showing the relation between PEP reactivity and mobilization of CCR5+ and CXCR2+ T cells.

although the sympathetic component of cardiac reactivity appears to be superior to overall reactivity (Cacioppo, 1994; Cacioppo et al., 1995). This is consistent with the present analyses. HR reactivity yielded a comparable pattern of results as presented in Fig. 1, albeit showing less pronounced differences. Indeed, comparing the high and low HR reactivity groups yielded significant group differences only for CD3+CCR5+ ($t_{(40)} = 2.66, p = .01$), CD3+CXCR3+ ($t_{(40)} = 2.15, p < .05$), and CD3+CXCR4+ ($t_{(40)} = 2.48, p < .05$). Finally, comparisons of high and low vagal/parasympathetic cardiac reactors (indexed by changes in RSA) yielded no statistical differences in regard to T cell responses (for all t tests, $t < 1.2, p > .3$).

Similar analyses as described above (i.e., dividing our sample in high vs low reactors based on median difference scores of PEP, HR, or RSA) were performed to explore monocyte responses in relation to cardiac and autonomic reactions. In contrast to the T lymphocytes, monocyte responses showed no significant associations with PEP reactivity, heart rate reactivity or vagal reactivity: for all t tests, $t < 1.5$ and $p > .2$.

4. Discussion

T lymphocytes and monocytes/macrophages are the most abundant cells found in the atherosclerotic plaque (Libby et al., 2002; Ross, 1999). These cells are attracted

towards the activated endothelium by chemokines that are initially secreted by the local endothelial cells. The present study investigated the effects of an acute stressor (public speaking) on the mobilization of T cells and monocytes that express receptors for these chemotactic factors. Whereas the total number of circulating CD3+ lymphocytes did not change, the speaking stressor induced an increase in CD3+ cells expressing receptors for chemokines that are known to be secreted by activated endothelium (GRO, NAP-2, ENA-78, IL-8, IP-10 for CXCR2, and CXCR3, RANTES for CCR5) (Burke-Gaffney et al., 2002; Kotani et al., 2002; Qi & Kreutzer, 1995; Seeger et al., 2002; Wang et al., 1998). No change was seen in the number of T cells carrying receptors for chemokines that are not secreted by inflamed endothelial cells (SDF-1 for CXCR4, LARC/MIP-3 α for CCR6).¹ The various monocyte subsets (as defined by chemokine receptor expression), on the other hand, did not show such specificity: all subsets increased in parallel with total monocyte numbers. Thus, the results indicate a selective mobilization of T cells that are sensitive to the chemotactic signals of an inflamed endothelium.

¹ Chemokine receptors are known to exhibit a remarkable pleiotrophism, and therefore it cannot be excluded that CXCR4 and CCR6 may show some responsivity to chemokines that are secreted by inflamed endothelial cells.

Ideographic analysis, comparing mobilization responses in subjects exhibiting high versus low sympathetic cardiac reactivity, confirmed and extended the results of nomothetic analyses. In line with previous research (Cacioppo et al., 1995; Uchino et al., 1995), it confirmed our assumption that sympathetic cardiac reactivity and immune reactivity are closely related phenomena. In particular, increases in T cell subsets were prominent in the high PEP reactors but were absent or even reversed for the low PEP reactors. For example, whereas the CD3+CCR6+ subset showed a trend towards increased cell numbers in high PEP reactors ($p = .08$), a significant decrease ($p < .01$) was observed in low PEP reactors (auxiliary analyses).

Ideographic analyses also indicated that the rapidly induced monocytosis is probably mediated by different mechanisms than the observed lymphocytosis. In contrast to the T lymphocytes, there were no significant associations between PEP reactivity and increases in monocyte numbers. The reasons for these disparate effects are unclear. Thus far, human studies have mainly focused on the effects of acute stress on lymphocyte subsets (Benschop, Rodriguez-Feuerhahn, & Schedlowski, 1996; Sanders & Straub, 2002), and further research into the acute redeployment of other major leukocyte populations (e.g., monocytes, neutrophils) seems warranted.

A long standing hypothesis in cardiovascular research, the so-called reactivity hypothesis, postulates that individuals who show exaggerated cardiovascular responses to mild acute stressors (like those encountered in everyday life) may be prone to the development of cardiovascular disease and acute cardiovascular syndromes (Kop, 1999; Krantz et al., 1996; Rozanski et al., 1999). The underlying assumption is that such everyday challenges cause wear and tear to the cardiovascular system due to a mobilization of resources (i.e., hemodynamic, endocrine, metabolic, and hemostatic) beyond metabolic demands (Cacioppo et al., 1998). Furthermore, as observed in the present study, acute stressors enhance immunosurveillance by lymphocytes that are primed to respond to activated endothelium. Consistent with the basic premise of the reactivity hypothesis, these responses appear to be particularly prominent in individuals that exhibited strong sympathetic cardiac reactions to stress. Acute psychological stressors may thus promote the migration of inflammatory cells to the sub-endothelia, hereby accelerating the atherosclerotic process and potentially contributing to the acute complications that follow stressful events. This presents a novel pathway, linking cardiac reactivity and immune reactivity with the development of cardiovascular disease. We may add that this model assumes the presence of an activated endothelium that guides migration of inflammatory cells that become mobilized during acute stress. Further elaborations of this model may be pro-

vided by research on the role of stress-induced migratory responses in acute cardiovascular complications such as plaque rupture.

T cells constitute a heterogeneous population, and it is well established that the CD8+ T cells in particular are mobilized during acute stress in humans (Benschop et al., 1996; Sanders & Straub, 2002). Hence, the selective increases observed in our study might reflect changes in this CD8+ sub-population. Examination of the literature suggests that this may only provide a partial explanation. For example, we found an increase in T cells that are positive for the receptors CXCR3 and CCR5, which are both highly expressed on CD4+ T cells (the Th1 subset in particular) (Qin et al., 1998). However, most studies find no change, or even a slight decrease, in CD4+ T cells in response to acute stress (Sanders & Straub, 2002). Conversely, CXCR4 is expressed on a large proportion of CD8+ T cells (Bleul et al., 1996; Oberlin et al., 1996), whereas CD3+CXCR4+ cell numbers were unaltered during the speaking stressor. Also, both the pleiotropic IL-8 receptor CXCR2 and the more specific IL-8 receptor CXCR1 are expressed on CD8+ cells, but not on CD4+ cells (Chuntharapai, Lee, Hebert, & Kim, 1994). Thus, if the observed increases in T cell sub-populations were simply to reflect increases in CD8+ T cells, then increases should have occurred in both CXCR1+ and CXCR2+ sub-population. Together these observations are in line with other reports showing a remarkable heterogeneity in chemokine receptor expression within various lymphocyte sub-populations, e.g., (Campbell et al., 1999; Campbell et al., 2001a, 2001b; Chuntharapai et al., 1994; Kunkel et al., 2002; Qin et al., 1998). It would therefore be interesting to investigate whether chemokine receptor expression might further differentiate CD4+ and CD8+ T cell subsets in regard to their response to acute stress (for complementary approaches see also; Mills, Goebel, Rehman, Irwin, & Maisel, 2000; Redwine, Snow, Mills, & Irwin, in press; Sanders & Straub, 2002).

We conclude that stress-induced cardiac sympathetic activation is associated with an environment of increased chemokine receptor-positive T cells, which, when coupled with endothelial activation, could support the basic atherosclerotic process of recruitment and inflammation.

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