

## Cellular and mucosal immune reactions to mental and cold stress: Associations with gender and cardiovascular reactivity

GONNEKE WILLEMSSEN,<sup>a</sup> DOUGLAS CARROLL,<sup>a</sup> CHRISTOPHER RING,<sup>a</sup>  
AND MARK DRAYSON<sup>b</sup>

<sup>a</sup>School of Sport and Exercise Sciences, University of Birmingham, UK

<sup>b</sup>Department of Immunology, University of Birmingham, UK

### Abstract

To examine gender differences in immune reactions to stress and relationships between immune and cardiovascular reactivity, measures of cellular and mucosal immunity and cardiovascular activity were recorded in 77 men and 78 women at rest and in response to active (mental arithmetic) and passive (cold pressor) stress tasks. Both tasks reduced CD4+ T cells and the CD4/8 ratio. Total lymphocytes, NK cells, CD8+ T cells, and secretory immunoglobulin A (sIgA) increased with active stress. Passive stress decreased sIgA. At rest, men had more NK cells, less CD4+ T cells, and fewer neutrophils than women. Mental stress increased sIgA in men but not women. Cardiovascular reactivity to active stress was associated with increases in NK cells. The data support the hypothesis that stress-related increases in lymphocytes are beta-adrenergically mediated, and suggest that the fall in CD4+ T cells may be alpha-adrenergically driven. Mechanisms underlying sIgA reactions are more difficult to determine. Men and women differed in some cell counts, but not in reactivity, although gender influenced sIgA reactions to arithmetic.

**Descriptors:** Cardiovascular reactivity, Cellular immunity, Cold pressor, Gender, Mental arithmetic, Mucosal immunity

There is evidence that standard laboratory stress tasks influence enumerative cellular immune measures. The most consistent result to emerge is an increase in circulating natural killer (NK) cells (Bachen et al., 1992; Cacioppo et al., 1995; Caggiula et al., 1995; Herbert et al., 1994; Matthews et al., 1995; Mills et al., 1995; Naliboff et al., 1991; Sgoutas-Emch et al., 1994). Although the evidence regarding other lymphocyte subsets is less consistent, the ratio of T helper (CD4+) cells to T suppressor/cytotoxic (CD8+) cells (CD4/8 ratio) has been observed to decrease in response to acute stress exposures, particularly in studies with larger sample sizes (Bachen et al., 1992; Cacioppo et al., 1995; Herbert et al., 1994; Matthews et al., 1995; Mills et al., 1995; Sgoutas-Emch et al., 1994). The rapidity of cellular immune reactions to acute laboratory stress suggests the possible involvement of the sympathetic nervous system. Supportive evidence is provided by studies showing that nonspecific adrenergic blockade attenuated stress-induced changes in NK cell numbers and the CD4/8 ratio (Bachen et al., 1995), and that epinephrine infusion and acute psychological stress appear to perturb cellular immunity in the same way (Crary

et al., 1983). Moreover, the finding that beta-adrenergic blockade diminished this increase in NK cell numbers (Benschop et al., 1994, 1996) implicates regulation by beta-adrenergic mechanisms. It might be hypothesized that stress-elicited changes in other enumerative cellular immune parameters are similarly beta-adrenergically driven. However, at present, the precise mechanism underlying changes in cellular immune parameters, other than NK cell numbers, remains to be established.

The majority of previous studies of cellular immune reactivity have focused on active stressors that elicit cardiovascular reactions characteristic of beta-adrenergic activation (e.g., increased cardiac contractility). It would seem equally important to study responses to passive stressors, such as the cold pressor test, which are characterized by alpha-adrenergic activation (e.g., increased peripheral resistance). Few studies have investigated the cellular immune response to passive stress tasks (Chi, Neumann, Mota-Marquez, & Dubberley, 1993; Delahanty et al., 1996; Eller, 1998; Zakowski, Cohen, Hall, Wollman, & Baum, 1994). Only two reported immune cell counts, both in response to cold pressor. Chi et al. found changes in the percentage of lymphocytes and neutrophils, although the direction of the change varied according to the resting baseline used. Eller (1998) reported increases in CD8+ T cell numbers. No study has directly compared enumerative cellular immune reactions to active and passive tasks. It was hypothesized that active stress would have a greater impact on cellular immunity than passive stress.

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Address reprint requests to: Gonneke Willemsen, Biological Psychology, Vrije Universiteit, Van der Boechorststraat 1, 1081 BT Amsterdam, The Netherlands. E-mail: ahm.willemsen@psy.vu.nl.

Acute psychological stress also influences mucosal immunity. Salivary sIgA has been found to increase in response to active laboratory stress tasks, such as mental arithmetic (Bosch et al., 2001; Carroll et al., 1996; Ring et al., 1999, 2000; Willemsen et al., 1998; Willemsen, Ring, McKeever, & Carroll, 2000; Winzer et al., 1999). Somewhat more mixed results have been found for passive stress. The cold pressor has been associated with decreased sIgA when measured during the task (Ring et al., 2000) but with increased sIgA when measured immediately after the task (Willemsen et al., 1998). Again, the rapidity of the sIgA response to stress suggests sympathetic nervous system involvement. However, neither alpha- nor beta-adrenergic blockade attenuated the sIgA increase to mental arithmetic (Ring et al., 2000; Winzer et al.). In addition, beta-blockade did not affect sIgA during cold pressor (Winzer et al.), although alpha-adrenergic blockade prevented the significant reduction in sIgA (Ring et al., 2000). Overall, these data suggest that cellular and mucosal immune responses are regulated differently. As yet only one study has measured both cellular and mucosal immune responses to stress (Kiecolt-Glaser et al., 1984); sIgA and cellular immune measures behaved differently under examination stress. Accordingly, it was hypothesized that salivary sIgA and cellular immune reactions to active and passive stress tasks would be poorly correlated.

Men and women have been reported to differ in their cardiovascular reactions to acute stress, with men exhibiting relatively larger vascular reactions and women exhibiting relatively larger cardiac reactions (Allen, Stoney, Owens, & Matthews, 1993; Girdler, Turner, Sherwood, & Light, 1990; Stoney, Davis, & Matthews, 1987). Given the linkages between the sympathetic nervous system and the immune system (Ader, Felten, & Cohen, 2000), such gender differences in alpha- and beta-adrenergic cardiovascular activation make it reasonable to hypothesize gender differences in immune reactions to stress. In addition, gender differences might also be expected on the grounds that many diseases involving immune dysregulation are more prevalent in women than men (Ansar-Ahmed, Penhale, & Talal, 1985). The few studies that have explored gender differences in immune reaction to acute stress report that men and women do not differ in either cellular reactions (Herbert et al., 1994; Kiecolt-Glaser et al., 1984; Mills et al., 1995) or sIgA reactions (Kiecolt-Glaser et al., 1984; Ring et al., 2000; Winzer et al., 1999). However, it is possible that these null findings reflect low power; studies have either suffered from small sample sizes (Herbert et al., 1994; Winzer et al., 1999; Ring et al., 2000) or a preponderance of one gender (Mills et al., 1995; Kiecolt-Glaser et al., 1984). In addition, previous studies have been limited to the use of active stress tasks, and it is also possible that gender differences will be more readily manifest in response to passive stress tasks. Accordingly, it was hypothesized that with a large sample size, and the inclusion of a passive (cold pressor) as well as an active (mental arithmetic) stress task, gender differences in cellular and mucosal immune reactions would be evident.

## Method

### Participants

One hundred and fifty-seven participants (78 men, 79 women) were recruited through advertisements at the University of Birmingham to take part in the study. For the men, the mean age was 25.21 years ( $SD = 6.42$ ), mean weight was 75.69 kg ( $SD = 9.76$ ), and mean height was 1.80 m ( $SD = 0.07$ ). For the women, the mean age was 26.57 years ( $SD = 8.01$ ), mean weight was 61.43 kg ( $SD = 7.70$ ), and mean height was 1.67 m ( $SD = 0.07$ ). On the day

of testing, none of the participants reported symptoms of upper respiratory tract infection. They were asked to refrain from consuming drinks containing caffeine 1 h prior to testing, and from physical exercise and drinking alcohol from the evening prior to testing. Participants were excluded if they suffered from pulmonary, cardiovascular, or immune disease, if they had taken prescribed medication or supplements in the previous 4 weeks, or if they smoked. Participants were paid.

### Cellular Immune Measurements

Using a separate venipuncture for each blood sample, blood was collected in 4.5 ml tubes (Vacutainer) containing ethylene diamine-tetraacetic acid (EDTA) to obtain cell counts. A full blood count, including the numbers of lymphocytes and neutrophils, was performed using a Hitechnicon cell counter by the Department of Haematology Clinical Laboratory (C.P.A. accredited), University Hospital Birmingham NHS Trust. Percentages of lymphocyte subsets were determined by flow cytometry in the Clinical Immunology Laboratory (C.P.A. accredited), Division of Immunity and Infection, University of Birmingham. A whole blood lysis method was used to stain the cells with the following pairs of FITC/phycoerythrin conjugated monoclonal antibodies (Becton Dickinson). Isotype matched control antibodies used were CD14/CD45, CD3/CD4 (CD4+ T cells), CD3/CD8 (CD8+ T cells), and CD57/CD56 (CD56+ indicating NK cells). Absolute numbers for each lymphocyte subset were determined by multiplying the percentage of these cells in the lymphocyte gate by the total lymphocyte count.

### Salivary sIgA Measurements

To determine saliva volume and sIgA concentration, unstimulated saliva samples were collected using cotton wool swabs (Salivettes, Sarstedt Ltd.). Before each sample, the participant swallowed to dry the mouth and then placed the swab underneath the tongue for 2 min. The participant then placed the swab in the plastic tube, which was sealed and frozen at  $-20^{\circ}\text{C}$  for later analysis. After thawing, saliva was extracted from the cotton by centrifugation at  $5 \times 10^3$  rpm for 5 min and, for each sample, saliva flow rate (in milliliters per minute) was obtained by weighing the amount extracted and assuming a specific gravity of one. The concentration of saliva (in micrograms per milliliter) was determined by a radial immunodiffusion (RID) assay (Bind A Rid, The Binding Site Ltd; for a detailed description see Winzer et al., 1999) by the Clinical Immunology Laboratory, University of Birmingham. The sIgA secretion rate (in micrograms per minute) was calculated as the product of sIgA concentration and saliva flow rate.

### Cardiovascular Measurements

Blood pressure was measured using an auscultatory sphygmomanometer (IBS-SD700, Industrial & Biomedical Sensors Corp.). The brachial cuff was placed around the left or right arm depending on which arm was used for venous blood sampling and the task undertaken: the cuff was placed on the arm contralateral to the arm where the most recent blood sample was obtained, thereby avoiding haematoma; and the cuff was placed on the nondominant arm during the mental arithmetic task. Measurements were initiated manually at fixed intervals throughout the session, and for each measurement, diastolic blood pressure (DBP) and systolic blood pressure (SBP) were recorded. Mean arterial pressure (MAP) was calculated using the formula:  $\text{MAP} = \text{DBP} + \frac{1}{3}(\text{SBP} - \text{DBP})$ .

Haemodynamic indices were recorded continuously throughout the rest and task periods using electrocardiography (ECG) and

impedance cardiography (ICG). The ECG was recorded using three spot electrodes (3M Health Care) in a modified chest configuration (Sherwood, Royal, Hutcheson, & Turner, 1992) by a cardiac monitor (509, Morgan Ltd.). The ICG was recorded using an impedance cardiograph (Minnesota Model 304B, Instrumentation for Medicine, Inc.) and four circumferential mylar band electrodes (Instrumentation for Medicine, Inc.). Recording electrodes were placed around the base of the neck and around the thorax at the level of the xiphisternal junction. The two current electrodes were placed parallel to the recording electrodes, at least 3 cm distal to the recording electrodes. The distance between the two measuring electrodes was measured at the front and the back, and the average of these two measurements was used in the calculation of stroke volume.

Using an interactive scoring program (Kelsey & Guethlein, 1990), the ECG and ICG signals were checked on a beat-to-beat basis, and the 60-s ensemble averages of the two signals were used to determine heart rate (HR), preejection period (PEP), left ventricular ejection time (LVET), and  $dZ/dt_{\max}$  relative to the B-wave. Stroke volume was determined using the Kubicek formula (Kubicek et al., 1974), and cardiac output (CO) was calculated as the product of HR and SV. Total peripheral resistance (TPR) was calculated using the formula  $TPR = (MAP/CO) \times 80$ .

#### **Cold Pressor and Mental Arithmetic Tasks**

In the 4-min cold pressor test, participants were required to keep their hand, up to the wristfold, immersed in 10°C water for 4 min (Willemsen et al., 1998). The 8-min mental arithmetic task used was the paced auditory serial addition test (PASAT) as adapted by Willemsen et al. (1998). Participants were presented with a series of single digit numbers and were required to add every number to the previously presented number. Numbers were presented by tape player and participants had to write their answers down. The task consisted of four consecutive 2-min series of 50, 60, 75, and 100 digits at presentation rates of 2.4, 2.0, 1.6, and 1.2 seconds respectively. Participants were told that the amount they could earn depended on task performance, but, in reality, payment was independent of performance.

#### **Procedure**

Upon arrival at the laboratory, the procedure was explained, demographic data were obtained, and the electrodes and blood pressure cuff were attached. To familiarize participants with the measurement protocol, a blood sample and a blood pressure reading were obtained but not saved. The participant then sat and formally relaxed for 10 min. Blood pressure was measured during Minutes 2, 6, and 10 of the rest period and a saliva sample was obtained during Minutes 9–10. Immediately after the rest period, a blood sample was obtained. The participant then performed the cold pressor and mental arithmetic tasks, separated by 10 min of rest; the order of completion of these two tasks was counterbalanced across participants. Blood pressure was measured at Minutes 2, 5, and 8 during the mental arithmetic task, and at Minutes 2 and 4 during cold pressor. In the last 2 min of each task, a saliva sample was obtained, and then immediately after each task, a blood sample was taken. The ECG and ICG signals were recorded continuously throughout the session. Following further rest, participants completed an 8-min exercise test (data not reported here).

#### **Data Reduction and Analysis**

For the cardiovascular data, averages were calculated for the first rest period and the tasks. For rest, the 60-s ensemble averages for

HR, PEP, and CO were averaged over Minutes 6–10, and the SBP, DBP, and MAP recordings for Minute 6 and Minute 10 were averaged. For the tasks, the 60-s ensemble averages for HR, PEP, and CO were averaged across the entire task period, and the blood pressure measurements for each task were averaged. The TPR values computed for the corresponding periods were averaged in a similar way. Thus, cardiovascular activity was reduced, for each parameter, to one value representing the initial rest and one value representing each task. The sIgA concentration and secretion rates were normalized using a base 10 logarithmic transformation prior to analysis.

Separate 2 (gender: men, women)  $\times$  3 (condition: rest, mental arithmetic, cold pressor) multivariate analyses of variance (MANOVAs) were conducted on each immune and cardiovascular variable. Wilks' lambda ( $\lambda$ ), the associated  $F$  and degrees of freedom are reported. Variations in the degrees of freedom reported reflect missing data. Eta-squared ( $\eta^2$ ), a measure of effect size, is also reported. Post hoc tests of conditions effects were performed using the Newman–Keuls method. Change scores were calculated by subtracting the rest value from the mental arithmetic and cold pressor values. Gender  $\times$  Condition interaction effects were explored using a 2 (gender: men, women) analysis of variance (ANOVA) applied to these change scores, separately for each task. Pearson correlation coefficients were computed on these change scores to examine the relationships between immune and cardiovascular reactivity, and cellular and mucosal immune reactivity.

## **Results**

### **Cellular Immunity**

The summary cellular immune data for rest, mental arithmetic, and cold pressor are presented separately for men and women in Table 1. MANOVAs yielded condition main effects for lymphocytes,  $\lambda = .832$ ,  $F(2, 107) = 10.82$ ,  $p < .0001$ ,  $\eta^2 = .064$ ; CD4+ T cells,  $\lambda = .810$ ,  $F(2, 107) = 12.75$ ,  $p < .00001$ ,  $\eta^2 = .099$ ; CD8+ T cells,  $\lambda = .884$ ,  $F(2, 107) = 7.02$ ,  $p < .001$ ,  $\eta^2 = .043$ ; CD4/8 ratio,  $\lambda = .652$ ,  $F(2, 107) = 28.54$ ,  $p < .00001$ ,  $\eta^2 = .203$ ; and NK cells,  $\lambda = .508$ ,  $F(2, 106) = 51.32$ ,  $p < .00001$ ,  $\eta^2 = .397$ , but not for neutrophils,  $\lambda = .983$ ,  $F(2, 106) = 0.92$ ,  $p > .40$ ,  $\eta^2 = .009$ . Post hoc analyses indicated that cell counts were higher with mental arithmetic than with rest and cold pressor for lymphocytes, CD8+ T cells, and NK cells. A different pattern emerged for helper T cells; the CD4+ T cell counts were reduced during both mental arithmetic and cold pressor relative to rest. Additionally, the CD4/8 ratio was lower in the mental arithmetic than the cold pressor condition, which, in turn, was lower than during rest. Gender main effects were found for CD4+ T cells,  $F(1, 108) = 8.55$ ,  $p < .005$ ,  $\eta^2 = .073$ ; NK cells,  $F(1, 107) = 7.27$ ,  $p < .01$ ,  $\eta^2 = .064$ ; and neutrophils,  $F(1, 107) = 8.38$ ,  $p < .005$ ,  $\eta^2 = .073$ . Women had more CD4+ T cells and neutrophils, and men had higher NK cell counts. There were no significant Gender  $\times$  Condition interaction effects.

### **Mucosal Immunity**

Table 1 also summarizes the salivary sIgA data for men and women. Condition effects emerged for sIgA concentration,  $\lambda = .651$ ,  $F(2, 124) = 33.23$ ,  $p < .00001$ ,  $\eta^2 = .218$ ; saliva flow rate,  $\lambda = .875$ ,  $F(2, 124) = 8.83$ ,  $p < .0005$ ,  $\eta^2 = .072$ ; and sIgA secretion rate,  $\lambda = .846$ ,  $F(2, 124) = 11.30$ ,  $p < .00005$ ,  $\eta^2 = .081$ . Post hoc tests indicated that sIgA concentration was higher during mental arithmetic than during rest, and that rest was higher than

**Table 1.** Mean (SD) Cellular and Mucosal Immune Values for Rest, Mental Arithmetic, and Cold Pressor for Men and Women

Variable	Rest	Mental arithmetic	Cold pressor
<b>Men</b>			
Lymphocytes ( $\times 10^6/l$ )	1577 (370)	1613 (370)	1550 (383)
CD4+ T cells ( $\times 10^6/l$ )	626 (170)	589 (143)	560 (144)
CD8+ T cells ( $\times 10^6/l$ )	419 (154)	423 (154)	405 (154)
CD4/8 ratio	1.63 (0.54)	1.51 (0.53)	1.63 (0.54)
NK cells ( $\times 10^6/l$ )	217 (87)	303 (137)	224 (106)
Neutrophils ( $\times 10^6/l$ )	2675 (826)	2751 (802)	2728 (916)
sIgA concentration ( $\mu\text{g/ml}$ )	225 (171)	404 (395)	179 (124)
Saliva flow rate (ml/min)	0.43 (0.31)	0.35 (0.22)	0.41 (0.29)
sIgA secretion rate ( $\mu\text{g/min}$ )	105 (114)	144 (188)	79 (88)
<b>Women</b>			
Lymphocytes ( $\times 10^6/l$ )	1579 (414)	1660 (403)	1569 (399)
CD4+ T cells ( $\times 10^6/l$ )	722 (230)	694 (203)	691 (211)
CD8+ T cells ( $\times 10^6/l$ )	446 (155)	473 (184)	448 (171)
CD4/8 ratio	1.74 (0.57)	1.60 (0.57)	1.66 (0.53)
NK cells ( $\times 10^6/l$ )	164 (70)	256 (107)	184 (77)
Neutrophils ( $\times 10^6/l$ )	3277 (1162)	3269 (1158)	3254 (1132)
sIgA concentration ( $\mu\text{g/ml}$ )	263 (266)	361 (376)	177 (177)
Saliva flow rate (ml/min)	0.39 (0.26)	0.29 (0.21)	0.36 (0.28)
sIgA secretion rate ( $\mu\text{g/min}$ )	114 (182)	91 (102)	77 (195)

cold pressor. Saliva flow was lower during mental arithmetic than during the other two conditions. The sIgA secretion rate was lower during cold pressor than during either rest or mental arithmetic. No main effects of gender were present. The Gender  $\times$  Condition interaction effect for sIgA secretion rate was marginal,  $\lambda = .956$ ,  $F(2, 124) = 2.87$ ,  $p = .06$ ,  $\eta^2 = .024$ . Supplementary 2 gender ANOVAs were performed on change scores (task minus rest). During mental arithmetic, sIgA secretion rate increased for men ( $M = 39.92 \mu\text{g/min}$ ) but not for women ( $M = -23.03 \mu\text{g/min}$ ),  $F(1, 125) = 5.60$ ,  $p < .05$ ,  $\eta^2 = .043$ . There were no differential effects of gender on reactions to the cold pressor,  $F(1, 125) = 2.62$ ,  $p > .10$ ,  $\eta^2 = .020$ , with both men ( $M = -25.32 \mu\text{g/min}$ ) and women ( $M = -38.31 \mu\text{g/min}$ ) showing reductions in sIgA secretion rate.

### Cardiovascular Reactivity

The summary data for the cardiovascular activity during the initial resting baseline, mental arithmetic, and cold pressor tasks are presented in Table 2. MANOVAs yielded Condition effects for all variables: SBP,  $\lambda = .398$ ,  $F(2, 150) = 113.53$ ,  $p < .00001$ ,  $\eta^2 = .370$ ; DBP,  $\lambda = .343$ ,  $F(2, 150) = 143.65$ ,  $p < .00001$ ,  $\eta^2 = .460$ ; HR,  $\lambda = .368$ ,  $F(2, 153) = 131.63$ ,  $p < .00001$ ,  $\eta^2 = .581$ ; PEP,  $\lambda = .535$ ,  $F(2, 153) = 66.52$ ,  $p < .00001$ ,  $\eta^2 = .398$ ; CO,  $\lambda = .327$ ,  $F(2, 152) = 156.72$ ,  $p < .00001$ ,  $\eta^2 = .377$ ; and TPR,  $\lambda = .368$ ,  $F(2, 148) = 126.97$ ,  $p < .00001$ ,  $\eta^2 = .274$ . Post hoc comparisons indicated that SBP and DBP were higher during cold pressor than during mental arithmetic, and greater during mental arithmetic than during rest. The HRs were faster and PEPs shorter during mental arithmetic than during the other two conditions, whereas COs were higher and TPRs lower during rest than during the other two conditions. Gender main effects were evident for SBP,  $F(1, 151) = 77.72$ ,  $p < .00001$ ,  $\eta^2 = .340$ , and HR,  $F(1, 154) = 9.38$ ,  $p < .005$ ,  $\eta^2 = .057$ ; women had lower SBPs but faster HRs than men. The only significant Gender  $\times$  Condition interaction effect was for PEP,  $\lambda = .932$ ,  $F(2, 153) = 5.62$ ,  $p < .005$ ,  $\eta^2 = .019$ . ANOVAs (2 genders) on the change scores showed that

women ( $M = -8.13 \text{ ms}$ ) and men ( $M = -8.83 \text{ ms}$ ) showed similar reactions to mental arithmetic,  $F(1, 154) = 0.19$ ,  $p > .66$ ,  $\eta^2 = .001$ ; however, women ( $M = -1.57 \text{ ms}$ ) and men ( $M = 0.95 \text{ ms}$ ) differed in their reactions to the cold pressor,  $F(1, 155) = 8.04$ ,  $p < .01$ ,  $\eta^2 = .049$ .

### Association between Immune and Cardiovascular Reactivity

Given that there were few gender differences in reactivity, the associations between changes in immune and cardiovascular variables were computed for the whole sample. Pearson correlations indicated that, for the mental arithmetic task, changes in NK cell numbers correlated with SBP,  $r(122) = .25$ ,  $p < .01$ , HR,  $r(125) = .37$ ,  $p < .00005$ , and PEP,  $r(125) = -.38$ ,  $p < .00001$ , and sIgA concentration changes correlated with SBP,  $r(129) = .20$ ,  $p < .05$ , and PEP,  $r(132) = -.22$ ,  $p < .05$ . Similar patterns of correlation

**Table 2.** Mean (SD) Cardiovascular Values for Rest, Mental Arithmetic, and Cold Pressor for Men and Women

Variable	Rest	Mental arithmetic	Cold pressor
<b>Men</b>			
SBP (mmHg)	124.5 (10.8)	133.3 (14.2)	135.2 (12.3)
DBP (mmHg)	73.7 (10.8)	81.4 (11.0)	85.5 (12.5)
HR (bpm)	64.7 (11.5)	75.5 (13.5)	63.5 (10.2)
PEP (ms)	107.9 (10.4)	99.1 (14.7)	108.8 (11.0)
CO (l/min)	8.13 (2.17)	7.10 (2.29)	6.88 (1.99)
TPR (d/s/cm <sup>-5</sup> )	983 (399)	1299 (819)	1313 (505)
<b>Women</b>			
SBP (mmHg)	111.0 (9.1)	117.0 (11.1)	122.8 (11.3)
DBP (mmHg)	73.6 (8.5)	78.9 (7.3)	83.6 (9.3)
HR (bpm)	69.1 (10.5)	81.3 (13.7)	69.0 (10.2)
PEP (ms)	106.8 (12.8)	98.7 (13.9)	105.4 (11.3)
CO (l/min)	8.0 (2.17)	7.04 (2.00)	6.94 (1.74)
TPR (d/s/cm <sup>-5</sup> )	908 (271)	1158 (587)	1203 (500)

coefficients emerged from supplementary analyses conducted separately for men and women. For the cold pressor, however, none of the coefficients was significant.

#### **Association Between Cellular and Mucosal Immune Reactivity**

Correlational analyses comparing cellular immune reactions and sIgA reactions to mental arithmetic and cold pressor, for whole sample or for men and women, yielded no significant coefficients.

### **Discussion**

#### **Immune Reactivity to Mental and Cold Stress**

As hypothesized, active stress had a greater impact on cellular immunity than passive stress. Mental arithmetic was associated with increases in overall lymphocyte numbers, circulating NK and CD8+ T cells. There was also a decrease in CD4+ T cells, which, along with the increase in CD8+ T cells, generated a significant reduction in the CD4/8 ratio. Far fewer effects emerged with the passive stress task, although the cold pressor was also associated with a reduced CD4/8 ratio, which, in this case, resulted exclusively from a decrease in CD4+ T cells. In contrast to the findings of a previous study (Eller, 1998), CD8+ T cells did not change in response to cold pressor. However, differences in the methodology of the two studies make direct comparisons difficult.

Active and passive stress tasks also had a different impact on mucosal immunity. Mental arithmetic elicited an increase in sIgA concentration, and although the associated sIgA secretion rate values were not significantly different from those recorded at rest, they were higher than those found during cold pressor. In contrast, both sIgA concentration and secretion rate decreased during cold pressor relative to rest. These findings for the cold pressor are broadly similar to the results from another recent study in our laboratory (Ring et al., 2000), although they contrast with nonsignificant change in sIgA found by Winzer et al. (1999) and the significant increase noted by Willemsen et al. (1998). Discrepancies in sIgA response to cold stress have been attributed to differences in the timing of saliva collection and to differences in the gender composition of the population studied (Ring et al., 2000). The present results are in line with the observations of Bosch et al. (2001) that sIgA increased to an active stress (a memory search task) but decreased to the passive stress of viewing a distressing video.

#### **Sympathetic Nervous System Mechanisms Underlying Immune Reactivity**

The cardiovascular data provide clues to the mechanisms underlying the observed stress-induced changes in immunity. Whereas mental arithmetic elicited changes suggestive of a mixed pattern of beta-adrenergic (shorter PEP) and alpha-adrenergic (increased TPR) sympathetic activation, the cold pressor provoked mainly alpha-adrenergic effects. Thus, the increases in NK and CD8+ T cells, exclusive to the mental arithmetic stress, can be reasonably attributed to beta-adrenergic effects. This conclusion is supported by the finding that propranolol attenuated the increase in NK cell numbers to an active stress, although in the case of CD8+ T cells, the beta-adrenoceptor antagonist exerted its main influence in the post stress recovery period (Benschop et al., 1994). Further, in the present study, NK cell number increases were significantly correlated with the extent of shortening of PEP. In contrast, because reduced CD4+ T cell numbers in the circulation were common to both the active and the passive stress, alpha-adrenergic mecha-

nisms could be involved. However, it should be conceded that Bachen et al. (1995) found no effects of labetalol, a combined alpha- and beta-adrenoceptor antagonist, on the CD4+ T cell response to an active stress protocol, although it did attenuate the stress-related reduction in the CD4/8 ratio. In the present study, reductions in the CD4/8 ratio were associated, as indicated, with both sorts of stress task.

The significant correlation between the increase in sIgA concentration and SBP and PEP during mental arithmetic suggests beta-adrenergic activation. Willemsen et al. (1998) also reported that PEP reactions were negatively associated in sIgA secretion rate on the second, but not the first, exposure to the mental arithmetic task. However, in a more recent study, beta-adrenoceptor blockade using propranolol did not moderate the increase in sIgA concentration seen with mental arithmetic (Winzer et al., 1999). Although the decrease in sIgA to cold pressor may be prevented by alpha-adrenoceptor blockade with doxazosin (Ring et al., 2000), there were no significant associations, in the current study, between TPR reactivity, an index of alpha-adrenergic activation, and sIgA reactions to cold stress.

The correspondence between sIgA and cellular immune responses to active and passive stress was, as expected, poor. Although there may be overlapping mechanisms, such as alpha-adrenergic activation, regulating cellular and mucosal immunity during exposure to cold stress, the absence of significant correlations between markers of cellular and mucosal immune reactivity argues that they are, for the most part, governed by different processes.

#### **Gender Differences**

Despite the large sample size of the study and the inclusion of both passive and active tasks, few significant gender effects were found. This suggests that power was not a limiting factor in detecting gender differences in earlier studies (Herbert et al., 1994; Kiecolt-Glaser et al., 1984; Mills et al., 1995; Ring et al., 2000; Winzer et al., 1999). Nevertheless, women had more circulating neutrophils and CD4+ T cells but fewer NK cells than men both at rest and during the tasks. Differences between men and women in resting levels of these parameters have been reported in a number of large scale immunological studies (Bain, 1996; Bain & England, 1975; Bryant et al., 1996; Huppert et al., 1998; Reichert et al., 1991). These gender differences in basal levels of neutrophils and lymphocyte subsets are substantial and important clinically; they need to be taken into account in assessing disease-related variation (Reichert et al., 1991). The only noteworthy gender difference in stress-induced immune reactivity appeared for sIgA secretion rate; mental arithmetic elicited an increase in sIgA secretion rate for men but a decrease for women. However, this gender difference did not appear for the passive stressor; both men and women showed a decrease in sIgA secretion rate during cold exposure. Accordingly, differences between studies in the direction of sIgA response to cold pressor (see Introduction and above) would not appear to be explained by gender.

#### **Summary**

Active mental stress had a more substantial and widespread impact on cellular immunity than passive cold stress. This finding may be attributed to greater beta-adrenergic activity during mental arithmetic than cold pressor. The most likely mechanisms for the increase in circulating NK and CD8+ T cells in response to mental arithmetic are adrenalin-mediated increases in blood flow (i.e., shear stress) and reductions in the adhesiveness of these cells to the

endothelium (Gleeson, 1994). Since the numbers of CD4+ T cells in the circulation decreased in response to both tasks, another mechanism is implicated. Although men and women differed at rest in a number of cellular immune parameters, there were no significant gender effects on reactivity. Salivary sIgA increased to the active stress but decreased to the passive stress, and men and

women differed in their sIgA secretion rate reactions to mental arithmetic. Cellular and mucosal immune reactions to stress were poorly correlated. Finally, it is clear from the effect sizes that, with the exception of NK cells and to a lesser extent the CD4/8 ratio, large sample sizes are required to detect mental and cold stress-induced immune reactions.

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