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Natural killer cell and proinflammatory cytokine responses to mental stress: associations with heart rate and heart rate variability

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Abstract

Associations between natural killer (NK) cell, proinflammatory cytokine stress responsivity, and cardiac autonomic responses (indexed by heart rate and heart rate variability) were assessed in 211 middle-aged men and women. Blood was drawn at baseline, immediately following color–word interference and mirror tracing tasks for the assessment of NK cell numbers, and 45 min post-stress for assessing plasma interleukin 6 (IL-6) and tumor necrosis factor alpha (TNFα) responses. Heart rate variability was measured as the root mean square of successive differences (RMSSD) in R–R intervals. Increases in NK cell counts following stress were positively associated with heart rate responses independently of age, sex, socioeconomic status, smoking, and change in hematocrit. Heart rate 45 min post-stress was positively associated with plasma IL-6 post-stress, and with TNFα changes from baseline, independently of covariates. No relationship between immune responses and heart rate variability was observed. We conclude that individual differences in sympathetically-driven cardiac stress responses are associated with NK and proinflammatory cytokine responses to psychological stress.

Keywords: Cardiac reactivity; Natural killer cells; Heart rate; Cytokines; Interleukin-6; Tumor necrosis factor alpha

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1. Introduction

The immune system is highly sensitive to acute psychological stress, with rapid changes in numbers of circulating natural killer (NK) cells and other lymphocytes, increased NK cell cytotoxicity, and reductions in mitogen-induced lymphocyte proliferation (Zorrilla et al., 2001). Increases in plasma levels of proinflammatory cytokines such as interleukin 6 (IL-6) and tumor necrosis factor alpha (TNFα) have also been described, although findings have been inconsistent (Ackerman et al., 1998; Dugué et al., 1993). Cytokine responses evolve more slowly than do other immune changes, so may not be observed in studies that are limited to immediate post-stress blood sampling (Steptoe et al., 2001).

It is probable that acute immune stress responses are mediated in part by sympathoadrenal activation. Primary and secondary lymphoid tissues are extensively innervated by the sympathetic nervous system (Elenkov et al., 2000). Catecholamines play a central role in lymphocyte migration, circulation, and trafficking (Benschop et al., 1996). NK cell counts are particularly sensitive to infusions of catecholamines, while T and B cell counts are less affected (Schedlowski et al., 1996). The effects of sympathoadrenal activity on inflammatory cytokines is more varied, with evidence of inhibition of plasma levels of TNFα (Elenkov et al., 2000; Goebel et al., 2000). However, Mohamed-Ali et al. (2001) have demonstrated that increases in IL-6 follow isoproterenol infusion in humans.

Positive associations between increases in norepinephrine and immune responses to stress have been described (Cohen et al., 2000; Mills et al., 1995a), but there are limitations to venous catecholamine sampling for the assessment of systemic sympathoadrenal activity (Hjemdahl, 1993). Methods such as microneurographic measurement of activity in sympathetic nerves (Wallin, 1981), and assessments of labeled norepinephine spillover (Esler et al., 1989) provide direct evidence, but are difficult to employ in larger scale studies. Cardiovascular variables are, therefore, frequently utilized as markers of sympathoadrenal activation. Systolic blood pressure, heart rate, and pre-ejection period all increase with sympathetic stimulation. Blood pressure is less valuable as a marker in this context than the other measures, since pressure responses tend to be preserved by alternative haemodynamic pathways when sympathetic stimulation is interrupted. Thus blood pressure stress responses are generally unaffected by β-adrenergic blockade, while heart rate responses are attenuated (Freyschuss et al., 1988). Lenders et al. (1988) found that heart rate responses to mental stress were reduced in adrenalectomised compared with intact participants, while pressure responses were no different. Julius (1988) has argued that there is haemodynamic plasticity in responses to autonomic activation, and that the cardiovascular system regulates the circulation to achieve blood pressure levels through alternative mechanisms if some are unavailable.

There is substantial evidence from laboratory studies that heart rate responses are associated with stress-induced increases in NK cell counts and activity (Benschop et al., 1998; Cohen et al., 2000; Manuck et al., 1991; Sgoutas-Emch et al., 1994). Conversely, NK cell mobilization and other immune responses to stress are reduced following α and β-adrenergic blockade (Bachen et al., 1992; Benschop et al., 1994).

There is little evidence concerning the cardiovascular correlates of inflammatory cytokine stress responses. However, we recently showed in a small study that IL-6 sampled 45 min post-stress and TNFα sampled 2 h post-stress were associated with cardiovascular responses (Steptoe et al., 2001). The first aim of this study was, therefore, to assess associations between heart rate and IL-6 and TNFα stress responses in a large sample of middle-aged men and women. Since cytokine responses to stress may take time to emerge, they were assessed from blood sampled 45 min post-stress. We also analyzed the relationship between heart rate and acute NK cell responses, as evidence from this age group is limited at present.

Heart rate is not of course a simple index of sympathetic activation, since it is regulated by the balance between sympathetic and parasympathetic pathways. Heart rate variability can, with caution, be regarded as an indicator of cardiac vagal control (Berntson et al., 1997). Consequently, we assessed heart rate variability as well as heart rate responses, reasoning that the combination would permit conclusions to be drawn about the relative importance of sympathetic and vagal influences on stress-induced immune changes. A number of other factors are potentially relevant to changes in plasma cytokines and NK cell counts, including hematocrit (Marsland et al., 1997), age, body mass, abdominal obesity, smoking, and method of blood sampling (Haack et al., 2002), so these were also included in the analyses.

These analyses were carried out on data from a study designed to assess psychobiological factors related to socioeconomic status. The responses to stress of heart rate, heart rate variability, cytokines, and NK cell counts in relation to socioeconomic status and sex have been described elsewhere (Steptoe et al., 2002a,b).

2. Method

2.1. Participants

These analyses were carried out on 211 of the 240 men and women who took part in mental stress testing as part of a larger study of psychobiological processes related to socioeconomic status. They were all members of the Whitehall II epidemiological cohort, a sample of 10 308 London-based civil servants recruited in 1985–1988 when aged 35–55 years to investigate demographic, psychosocial and biological risk factors for CHD (Marmot et al., 1991). Participants were of Caucasian origin, aged 45–59 years, with no history of coronary heart disease, no cancer in the previous 5 years, no previous diagnosis or treatment for hypertension, and no treatments for psychiatric illness or endocrine conditions. TNFα results were obtained from all 211 participants and IL-6 from 210, but for reasons of cost, NK cell counts were available from only 105 individuals. These 105 were drawn at random from the larger study, within the constraints that the grade of employment and sex distribution were matched to the sample as a whole.

2.2. Measures

Body weight, height, waist and hip circumference were measured using standardized methods. Heart rate were monitored continuously as pulse rate from the finger using a Portapres-2, a portable version of the Finapres device that shows good reproducibility and accuracy in a range of settings (Castiglioni et al., 1999; Imholz et al., 1993). Heart rate variability was assessed as the root mean square of successive differences (RMSSD) in R–R intervals obtained from a 3-lead electrocardiogram using an ambulatory cardiac impedance device (VU-AMS, Free University, Amsterdam, NL; de Geus et al., 1995). This measure was not available for a block of participants owing to equipment breakdown, so results were analyzed from 159 participants. Blood was collected in EDTA tubes and centrifuged immediately at 2500 rpm for 10 min at room temperature. The plasma was removed and stored at −80 °C until analysis. TNF-α and IL-6 were measured using high sensitivity two-site ELISAs from R and D Systems (Oxford, UK). The limit of detection of the human TNFα assay was 0.10 pg/ml with intra- and inter-assay coefficients of variation (CVs) of 6.9 and 8.4%. For IL-6, the limit of detection was 0.09 pg/ml, and intra- and inter-assay CVs were 5.3 and 9.2%. NK cells were assessed using a Becton–Dickinson FACScan flow cytometer (Oxford, UK) in whole blood using TriTEST™ fluorescein isothiocyanate (FITC) phycoerythrin (PE) and peridinin chlorophyll protein (PerCP) three-colour direct immunofluorescence reagents and TRU-COUNT™ absolute count tubes. Cells expressing CD16CD56 (NK cells) were enumerated, and outliers were excluded using the Becton–Dickinson reference ranges. Hematocrit was assessed immediately after each blood sample was drawn using a micro-hematocrit centrifuge and reader (Hawksley Gelman, Lancing, Sussex, UK).

2.3. Mental stress tests

Mental stress was induced by two behavioral tasks. The first was a computerized colour–word interference task, involving the successive presentation of target color words (e.g. green, yellow), printed in another color (Muldoon et al., 1992). The task was to press a computer key that corresponded to the position at the bottom of the screen of the name of the color in which the target word was printed, and the rate of presentation of stimuli was adjusted to the performance of the participant, to ensure sustained demands. The second task was mirror tracing, involving the tracing of a star with a metal stylus which could only be seen in mirror image (Owens et al., 1993).

2.4. Procedure

Testing was carried out both in the morning and afternoon in a light and temperature controlled laboratory. Participants were instructed not to have drunk tea, coffee, or caffeinated beverages, or to have smoked for at least 2 h prior to the study, and not to have consumed alcohol or have exercised strenuously on the

evening before or the day of testing. Individuals who had taken over-the-counter anti-inflammatory or antihistamine medication over the previous 2 days were rescheduled. The study was approved by the UCL/UCLH Committee on the Ethics of Human Research.

Following instrumentation for the measurement of cardiovascular variables, a venous cannula was inserted for the periodic collection of blood samples, and the participant rested for 30 min. Baseline heart rate was recorded for the last 5 min, and heart rate variability for the last 10 min this period. A baseline blood sample was drawn, and the two 5 min tasks were then administered in counterbalanced order. Heart rate and heart rate variability were monitored continuously throughout the tasks. A second blood sample was drawn immediately after tasks, and a third 45 min later. Heart rate was monitored for min 40–45 post-stress, and heart rate variability for min 30–40. Participants rested quietly during the post-stress period.

In 54 participants, the venous cannula was not successfully inserted immediately. So as to avoid the additional stress associated with finding other suitable veins, blood sampling in these individuals was carried out by repeated venepuncture. In a further 38 participants, the intravenous line did not remain patent through the post-task period, so later blood samples were obtained through separate venepunctures. The possible impact of these variations on NK and cytokine responses was taken into account in the analysis.

2.5. Statistical analysis

Heart rate was derived from the pulse intervals recorded with the Portapres, and was averaged into four 5 min periods (baseline, task 1, task 2, and 40–45 min post-stress). Heart rate variability was averaged into 10 min baseline and post-stress periods, and two 5 min task periods. The data for task periods were subsequently averaged to produce a single value for stress for each variable. NK cell counts and hematocrit were analyzed from all three blood samples, and IL-6 and TNFα from the baseline and 45 min samples only.

Changes in heart rate, heart rate variability, NK cell counts, cytokines and hematocrit across the study were assessed using repeated measures analysis of variance. The Greenhouse–Geisser correction was applied where appropriate, and post hoc tests were conducted with the LSD method. Heart rate and heart rate variability were categorized into quartiles for assessing associations with NK cells and cytokines. Quartiles were constructed for baseline levels (for assessing associations with baseline NK cell counts and cytokines), heart rate and heart rate variability responses to tasks (associations with NK cell stress responses), and 45 min post-stress values (associations with cytokine stress responses). Analysis of covariance was employed, with heart rate or heart rate variability quartile as the between-subject factor, and P values for linear trends across categories were computed (since we did not predict non-linear effects). Since we have previously shown differences either at baseline or in stress responses associated with sex and grade of employment, these variables were included along with age and hematocrit as covariates in all analyses. Additional covariates were introduced if they related to

NK cell counts or cytokine levels, as described in the results (Section 3.1). Additionally, logistic regressions were carried out on IL-6 and TNFα, assessing the odds of increases versus decreases or no change in cytokine levels following stress, in relation to heart rate and heart rate variability.

3. Results

The background characteristics of the men and women in this study are summarized in Table 1. Men were slightly older on average than women ($F(1,209) = 5.44$, $P = 021$). There were no sex differences in the distribution of participants by grade of employment, cigarette smoking, or body mass index. Waist/hip ratios were greater in men than women ($F(1,209) = 73.8$, $P < 0.001$), as is generally found in the literature.

The heart rate, heart rate variability, NK cell and inflammatory cytokine responses to mental stress have been detailed elsewhere, but are briefly summarized in Table 2 (Steptoe et al., 2002a,b). Heart rate changed across periods ($F(2,396) = 280.2$, $P < 0.001$), with an average increase of 6.0 bpm during stress. Post hoc tests indicated that heart rate 45 min post-stress was lower than at baseline. The heart rate increase was associated with a reduction in heart rate variability between baseline and stress periods, with a subsequent increase in variability 45 min later ($F(1,143) = 6.88$, $P = 0.01$). NK cell counts changed substantially over periods ($F(2,178) = 42.9$, $P < 0.001$), increasing with stress and declining during the post-stress period. However, NK cell counts 45 min post-stress remained above baseline levels. There was an increase in IL-6 concentration ($F(1,199) = 13.5$, $P < 0.001$), with 61.2% of participants having higher values 45 min post-stress than at baseline. In the case of TNFα, the difference between samples was not significant ($F(1,209) = 3.40$, $P = 0.067$), but increases were recorded in 54% of participants, with 46% showing no change or decreases in concentration. Finally, there was a reliable increase in hematocrit with stress, followed by a reduction to levels that still remained above

Table 1
Demographic and physical characteristics of participants

	Men ($n = 117$)	Women ($n = 94$)
Age (years)	52.6 ± 2.7	51.7 ± 2.7*
Grade of employment		
Higher (%)	40.1	36.2
Intermediate (%)	32.5	34.0
Lower (%)	27.4	29.8
Cigarette smokers (%)	12.1	7.4
Body mass index	25.8 ± 3.3	25.4 ± 3.9
Waist/hip ratio	0.906 ± 0.07	0.802 ± 0.11**

Mean ± standard deviation (S.D.) and percentages. Differences between sexes * = $P < 0.05$; ** = $P < 0.001$.

Table 2
Cardiovascular, NK cell, and cytokine responses to stress

	Baseline	Stress	45 min post-stress
Heart rate (bpm)	64.9 ± 9.0[a]	71.9 ± 10.1[b]	63.2 ± 8.2[c]
Heart rate variability (RMSSD in ms)	29.2 ± 13.6[a]	26.9 ± 13.5[b]	35.3 ± 15.5[c]
NK cells (cells per mm^3)	194.1 ± 72[a]	273.7 ± 108[b]	229.7 ± 100[c]
IL-6 (pg/ml)	1.26 ± .77[a]		1.35 ± .85[b]
TNFα (pg/ml)	2.35 ± 1.1		2.42 ± 1.1
Hematocrit (%)	39.2 ± 3.1[a]	39.9 ± 3.1[b]	39.7 ± 3.0[c]

Mean ± S.D. values in each row with different superscripts are significantly different from each other ($P < 0.05$).

baseline at 45 min post-stress ($F(2,408) = 24.0$, $P < 0.001$). As noted elsewhere, these physiological responses were associated with increased subjective stress, and tasks were rated as challenging, involving, and uncontrollable (Steptoe et al., 2002a).

3.1. Factors associated with NK cell and cytokine responses

The potential influence of time of day, season of the year, age, body mass index, waist/hip ratio, smoking status, method of blood sampling, and hormone replacement therapy in women, on NK cell counts and cytokine concentrations was assessed prior to the main analyses. NK responses were not related to any of these factors except for smoking status, with larger responses in smokers than non-smokers. The NK analyses, therefore, included age, sex, grade of employment, hematocrit and smoking as covariates.

Resting IL-6 concentration was positively associated with body mass index and waist/hip ratio, after controlling for sex, while responses to stress differed by method of blood sampling. Age, sex, grade of employment, body mass index, waist/hip ratio, and method of blood sampling were, therefore, included as covariates. By contrast, TNFα was not associated with factors apart from age, sex, grade of employment, and hematocrit, so these were included as covariates.

3.2. Natural killer cell responses and heart rate

NK cells counts at baseline were unrelated to heart rate. Comparison was made between NK cell responses to stress according to quartiles of heart rate stress responses. The mean heart rate changes for the four quartiles were −0.4, 4.5, 8.2, and 14.4 bpm. There was a main effect for heart rate stress response quartile in NK cell count changes between baseline and stress samples, after adjusting for age, sex, grade of employment, smoking, and change in hematocrit ($F(3,92) = 3.33$, $P = 0.005$). This result is shown in Fig. 1, where it is evident that the change in NK cell number in response to stress was greater in participants with larger heart rate stress responses. Post hoc tests indicated that the highest heart rate quartile differed

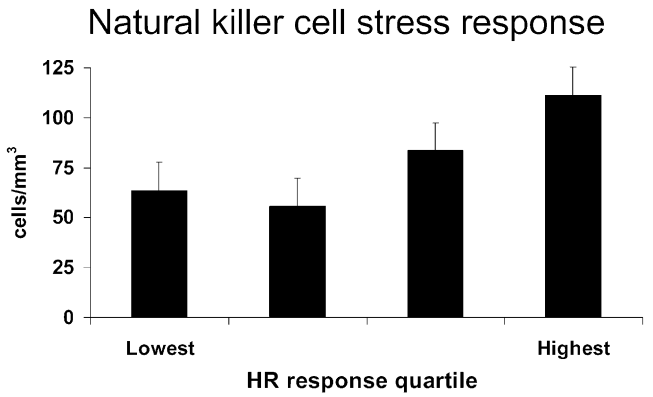

Fig. 1. Mean difference in NK cell number between baseline and stress samples, as a function of quartiles of heart rate stress response. The mean increase in heart rate from baseline to stress was 14.4 bpm in the highest, falling to −0.4 bpm in the lowest quartile. Values are adjusted for age, sex, grade of employment, smoking, and change in hematocrit. Error bars are S.E.M.

from the lowest and second quartiles ($P < 0.01$). There were no significant associations between NK cell counts or responses and heart rate variability.

3.3. Cytokine responses and heart rate

Neither the IL-6 concentration at baseline nor the change in IL-6 following stress was related to heart rate. However, IL-6 levels obtained 45 min post-stress were related to heart rate at this time point. The mean heart rates in the four quartiles of data measured 40–45 min post-stress were 52.2, 60.6, 65.5, and 73.6 bpm. After covarying for age, sex, grade of employment, body mass index, waist/hip ratio, hematocrit, method of blood sampling, and baseline heart rate, there was a significant linear trend across HR quartiles in the post-stress IL-6 concentration ($F(3,189) = 3.11$, $P = 0.029$). As can be seen in Fig. 2, the IL-6 concentration post-stress was substantially greater in the highest compared with the lowest heart rate quartile. In post hoc tests, IL-6 concentration in the lowest quartile differed from that in the second and highest quartiles, and values in the third and fourth quartiles also differed significantly ($P < 0.05$). Interestingly, baseline Il-6 levels were also positively associated with HR measured 45 min post-stress ($F(3,188) = 3.46$, $P < 0.019$), independently of covariates. This raises the possibility that baseline IL-6 influences heart rate recovery following stress, rather than the reverse, a point reviewed in the Section 4.

The likelihood of responding to mental stress with an increase rather than a reduction or no change in IL-6 was also related to heart rate levels 45 min post-stress. Compared with the lowest heart rate quartile, the odds ratio for showing an increase in IL-6 was 2.80 (95% confidence intervals 1.09–7.18) for individuals in the highest quartile, adjusted for age, sex, grade of employment, body mass index, waist/hip ratio, hematocrit, and method of blood sampling. When baseline IL-6 was

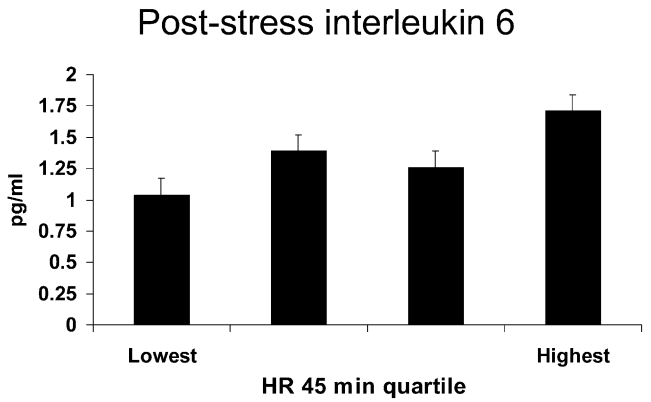

Fig. 2. Mean interleukin six concentration post-stress as a function of quartiles of heart rate 40–45 min post-stress. The mean heart rate in the highest quartile was 73.6 bpm, falling to 52.2 bpm in the lowest quartile. Values are adjusted for age, sex, grade of employment, body mass index, waist/hip ratio, method of blood sampling, baseline heart rate and hematocrit. Error bars are S.E.M.

included in the model, the odds ratio for an increase in IL-6 following stress in the highest quartile group rose to 3.31 (95% confidence intervals 1.25–8.82).

The concentration of TNFα was not associated with heart rate at baseline. Heart rate levels 45 min post-stress were positively related to TNFα sampled after stress ($F(1,192) = 3.61$, $P = 0.007$). In addition, changes in TNFα across the session were associated with heart rate levels 45 min post-stress, independently of age, sex, grade of employment, change in hematocrit, and baseline heart rate ($F(1,191) = 3.78$, $P < 0.001$). The mean change in TNFα in the lowest heart rate quartile averaged -0.16 pg/ml, compared with $+0.34$ pg/ml in the highest quartile (Fig. 3). As in the analysis of IL-6, the likelihood of an increase in TNFα with stress was related to heart rate 45

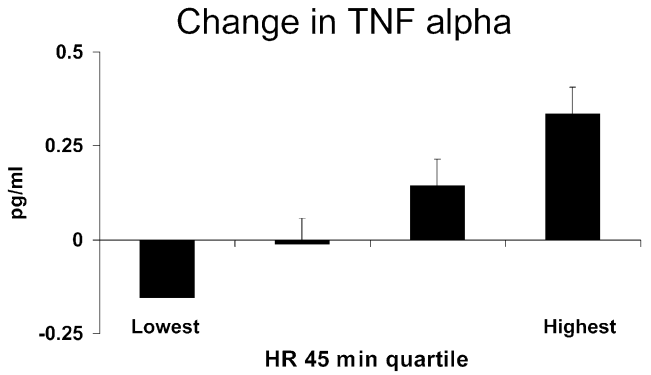

Fig. 3. Mean change in TNFα between baseline and post-stress, as a function of quartiles of heart rate 40–45 min post-stress. Values are adjusted for age, sex, grade of employment, baseline heart rate and hematocrit. Error bars are S.E.M.

min post-stress, with an odds ratio of 2.63 (95% confidence intervals 1.14–6.11) for the highest compared with the lowest quartile, adjusted for covariates.

No associations were observed between heart rate variability and cytokines either at rest or following stress.

4. Discussion

The results of these analyses indicate that NK cell and inflammatory cytokine responses to psychological stress were associated with heart rate but not heart rate variability. We categorized heart rate and heart rate variability into quartiles rather than using a continuous regression approach, so as to be able to investigate effects on a common metric. However, the results were similar when regression methods were used. No relationships between resting heart rate and baseline NK cell counts or IL-6 and TNFα were observed, but associations did emerge when values after stress were analyzed. NK cell counts rose acutely during stressful tasks, and the increase was positively related to heart rate responses. The rise in number of circulating NK cells was almost twice as great among participants in the highest compared with lowest heart rate response quartile (Fig. 1). The change from baseline in TNFα was positively associated with heart rate recorded 45 min post-stress, while IL-6 levels but not changes were related to heart rate. These effects were independent of covariates including baseline heart rate. They were also maintained after controlling for hematocrit, suggesting that stress-induced changes in hemoconcentration were not responsible for the findings. A sizable proportion of individuals did not show any increase in IL-6 or TNFα following stress. Logistic regressions indicated that the likelihood of a cytokine increase was again associated with heart rate independently of covariates. The absence of any relationship with heart rate variability suggests, but does not confirm, that individual differences in sympathetic rather than parasympathetically driven stress responsivity were associated with the immune activation process.

Relationships between cardiovascular reactivity and NK cell stress responses have been recorded in several previous studies. However, the data are not consistent, since changes in NK cell numbers have been associated with heart rate reactions in some studies (Benschop et al., 1998; Cohen et al., 2000; Mills et al., 1995b), but not others (Sgoutas-Emch et al., 1994). Most research has involved young, healthy groups (Cohen et al., 2000; Matthews et al., 1995; Sgoutas-Emch et al., 1994; Uchino et al., 1995), and studies of older samples have been limited to small samples (Naliboff et al., 1991). The present findings, therefore, add to the literature in demonstrating that heart rate reactivity is positively associated with increases in NK cell counts in middle-aged working men and women. The rapid responses are consistent with the impact of adrenaline infusion on NK cell numbers, which peak within 10 min (Schedlowski et al., 1996).

There is growing interest in the involvement of inflammatory cytokines in stress responses and the development of depression, coronary heart disease, and autoimmune disorders (Anisman and Merali, 2002; Black and Garbutt, 2002;

Jacobs et al., 2001). Effects of stress on mitogen-stimulated cytokine production have been described (Ackerman et al., 1998; Dugué et al., 2001; Goebel et al., 2000), but there have been few studies of plasma levels (Dugué et al., 1993; Heesen et al., 2002). In our previous study, we found associations between cytokine responses and blood pressure as well as heart rate (Steptoe et al., 2001). We limited analysis to heart rate and heart rate variability in the present investigation, since blood pressure effects are more difficult to interpret, and we were concerned about the dangers of multiple comparisons. The associations between cytokine stress responses and heart rate recorded 45 min post-stress are interesting in view of the fact that by this stage of the study, average heart rate had fallen below baseline (Table 2). It appears that individual differences in post-stress adaptation were particularly important markers of psychophysiological responsivity. This is consistent with the models of allostatic load postulated by McEwen (1998), in which failures of post-stress restitution of homeostasis may be particularly significant pathologically.

Circulating IL-6 derives from activated leukocytes, fibroblasts, endothelial cells and adipose tissue (Mohamed-Ali et al., 1997). It is involved in endothelial dysfunction, the expression of adhesion molecules, and in platelet activation. TNFα has several functions in inflammation, promoting leukocyte adhesion and migration, regulating macrophage activation, and modulating lymphocyte development. In atherogenesis, TNFα promotes T-cell activation, foam cell formation, and induces macrophage colony stimulating factor (Glass and Witztum, 2001). In advanced coronary artery disease, vulnerable plaques contain activated T-cells that express TNFα and IL-6, which in turn stimulate extracellular collagen matrix production and activate macrophages. Population studies have demonstrated that plasma IL-6 predicts future mortality in healthy men and women (Ridker et al., 2000a,b; Volpato et al., 2001). The concentration of IL-6 and TNFα in patients with unstable angina on admission to hospital is positively associated with risk of in-hospital cardiac events and subsequent mortality (Biasucci et al., 1999; Lindmark et al., 2001). Plasma IL-6 and TNFα have also been associated with depression (Dentino et al., 1999), and with the development of disability in old age (Ferrucci et al., 1999). The present findings suggest that individual differences in heart rate responses following stress may be associated with small elevations in these proinflammatory cytokines, which may in turn promote risk of disease and disability.

The interpretation of the association between heart rate and IL-6 is not as clear-cut as those involving NK cells or TNFα. The reason is that IL-6 levels post-stress but not changes from baseline were associated with heart rate sampled at 45 min. Additionally, IL-6 at baseline was related to heart rate at 45 min, but not with heart rate at baseline. This result raises the possibility that baseline IL-6 drove stress-induced changes in heart rate, rather than the reverse. In a study involving artificial administration of IL-6, Torpy et al. (2000) observed increases in heart rate after 90 min in healthy volunteers and after 30 min in women suffering from fibromyalgia, indicating that IL-6 may modulate sympathetic activation or cardiac β-receptor sensitivity. On the other hand, the likelihood of an increase in IL-6 post-stress was related to heart rate at 45 min independently of baseline IL-6, consistent with a

functional role of cardiac activation. Both processes may be operating, and the present experimental design was not able to distinguish these possibilities.

The limitations of this study should be acknowledged. Data were collected from a sample of white middle-aged men and women, and results may not generalize to other groups. Heart rate was used as a marker of sympathetically-driven cardiovascular activation, but pre-ejection period would have been valuable in this context (Uchino et al., 1995). Unfortunately, signal processing difficulties limited the cardiac impedance data available for analysis to a subsample of the complete cohort. In addition to the sympathoadrenal pathways investigated here, cortisol and the activity of the hypothalamic–pituitary–adrenocortical axis are related to cytokine production (Webster et al., 2002). We have addressed this aspect elsewhere, and observed that IL-6 but not TNFα levels were inversely associated with post-stress cortisol (submitted for publication). Adding post-stress cortisol as a covariate to the present analyses did not alter the pattern of results, so findings related to heart rate were evidently independent. Finally, financial constraints prevented us from analyzing NK cells on the complete cohort, and from assessing NK cell activity. Nevertheless, the strong associations between heart rate and NK cell and cytokine levels following stress suggests that changes in inflammatory and immune processes may provide a further mechanism through which cardiovascular reactivity influences disease risk.

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