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Rapid Changes in Cellular Immunity Following a Confrontational Role-Play Stressor[1]

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Recent laboratory studies have shown several immune system changes consistently associated with brief stress including increases in circulating natural killer (NK) cell numbers, increases in NK cell cytotoxicity (NKCC), increases in suppressor cytotoxic (CD8) T cell numbers, and decreases in the in vitro proliferative response to mitogen stimulation. In the present study, we use a confrontational role-play, which brings out responses varying from assertion to capitulation and examine the psychological, behavioral, physiological, and immune system responses to this task compared to a resting control task. Compared to the control condition, the brief confrontational role-play led to significant subjective and physiological arousal and increases in circulating NK (CD16, CD56) as well as large granular lymphocyte (CD57) cells and suppressor/cytotoxic T cells (CD8). There were also significant relationships between stress-related increases in the cardiovascular measures and the numbers of circulating NK cells. These findings support sympathetic nervous system activation as a primary mechanism for increases in NK cell numbers under challenge. These role-play results are generally consistent with those from other laboratory tasks such as mental arithmetic. However, in contrast to previously examined brief stressors, the role-play led to decreased NKCC adjusted for percentage of NK cells. This apparent differential change in NK cytotoxicity across different types of activating experimental tasks points to the importance of examining dimensions of the behavioral and emotional response to challenge or threat in addition to that of autonomic arousal.

INTRODUCTION

There is a small but growing literature examining the effect of psychological variables on the immune system using brief, stressful laboratory tasks. In 1992 Kiecolt-Glaser, Cacioppo, Malarkey, and Glaser (1992) reviewed nine initial studies and noted several consistent immune changes associated with brief stress including increases in circulating natural killer (NK) cell numbers, increases in suppressor cytotoxic (CD8) T cell numbers and decreases in the *in vitro* proliferative response to mitogen stimulation, particularly with concanavalin A. In general, the immune changes lasted the duration of the stressor but returned to baseline shortly afterward. They also found evidence in these studies for greater immune changes with greater arousal, as indexed by cardiovascular measures. More recent studies of immune measures before and after brief laboratory tasks generally confirm these observations (Benschop, Brosschot, Godaert, de Smet, Geenen, Olff, Heijnen, & Ballieux, 1994a; Benschop, Nieuwenhuis, Tromp, Godaert, Ballieux, & van Doornen, 1994b; Cacioppo, Malarkey, Kiecolt-Glaser, Uchino, Sgoutas-Emch, Sheridan, Berntson, & Glaser, 1995; Futterman, Kemeny, Shapiro, & Fahey, 1994; Herbert, Cohen, Marsland, Bachen, Rabin, Muldoon, & Manuck, 1994; Kiecolt-Glaser, Malarkey, Chee,

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Newton, Cacioppo, Mao, & Glaser, 1993; Naliboff, Solomon, Gilmore, Benton, Morley, & Fahey, 1995; Schedlowski, Jacobs, Stratmann, Richter, Hadicke, Tewes, Wagner, & Schmidt, 1993; Stone, Valdimarsdottir, Katkin, Burns, Cox, Lee, Fine, Ingle, & Bovbjerg, 1993; Zakowski, Cohen, Hall, Wollman, & Baum, 1994). In addition to the finding of increased NK cell numbers, several of the recent studies have shown increases in NK cell cytotoxicity (NKCC) with brief moderate to intense stress, and two studies indicate that, under some circumstances, NKCC and NK numbers may rebound below baseline values after an acute stress (Brosschot, Benschop, Godaert, de Smet, Olff, Heijnen, & Ballieux, 1992; Schedlowski et al., 1993).

The changes in immune measures observed in these studies may be viewed as part of the adaptive response to acute threat and, perhaps, preparation for injury. The rapidity of changes (in as little as 5 min after initiation of a stressor) and the similarity of many of the findings to those from an infusion of epinephrine suggest sympathetically mediated selective demargination of subclasses of lymphocytes as a primary mechanism for the response (Crary, Hauser, Borysenko, Kutz, Hoban, Ault, Weiner, & Benson, 1983; Kappel, Tvede, Galbo, Haahr, Kjaer, Linstow, Klarlund, & Pedersen, 1991; Tonneson, Christensen, & Brinklov, 1987). Increased blood flow coupled with changes in the expression of adhesion molecules on the surface of different subsets of lymphocytes may underlie selective redistribution (Benschop, Oostveen, Heijnen, & Ballieux, 1993). Although sympathetic arousal is common to the stressors studied, other dimensions of the stress response related to the type of stressor or individual difference variables may also influence the pattern of immune changes. We found, for example, that physically healthy older and younger subjects had similar psychological and cardiovascular responses to mental arithmetic and also showed similar increases in numbers of NK and CD8 cells (Naliboff, Benton, Solomon, Morley, Fahey, Bloom, Makinodan, & Gilmore, 1991). However, only younger subjects showed increases in NKCC following the stressor, indicating a different pattern of immune response with age. Sieber, Rodin, Larson, Ortega, and Cummings (1992) report data from a study which manipulated perceived control through success or failure in turning off a stressor (loud noise). They found decreased NKCC (opposite to what is usually reported for acute stress) only in a control group which had no ability to respond at all to the stressor. Futterman et al. (1994) found expression of both positive and negative moods by method actors led to increases in NK numbers and NKCC but the moods differentially affected response to the mitogen PHA. Thus, while standard stressors yield many consistent immune changes, which seem to result from intensity of activation, there is some evidence that the pattern of immune changes in response to stress may be significantly different depending on individual difference variables, quality of stress, and, perhaps, response options. Two *in vitro* functional immune measures, NKCC and proliferative response to T cell mitogen stimulation, may be particularly sensitive to stressor dimensions other than activation. An important question is whether the variability in NKCC response (or other immune measures) across laboratory experiments can help explain the often opposite results found between human laboratory studies and the descriptive studies of naturalistic life stress or studies of experimental stress in animals. For example, life stress in humans and experimental stress in animals appear to result in decreased NKCC while human laboratory stress may lead to increased NKCC. (Cunnick, Lysle, Armfield, & Rabin, 1988; Glaser, Rice, Speicher, Stout, & Kiecolt-Glaser, 1986; Herbert, & Cohen, 1993; Kiecolt-Glaser, Glaser, Strain, Stout, Tarr, Holliday, & Speicher, 1986; Lewis, Shavit, Martin, Terman, Gale, & Liebeskind, 1986; Morrow-Tesch, McGlone, & Norman, 1993; Naliboff et al., 1991; Schedlowski et al., 1993; Schlesinger & Yodfat, 1991; Vollhardt, 1991; Zalcman, Irwin, & Anisman, 1991).

As indicated by the Sieber et al. (1992) and Futterman et al. (1994) data, alternative laboratory paradigms may be needed further to test hypotheses regarding how differences in emotional quality and behavioral options effect the immune response. Standard stress tasks (such as Stroop or mental arithmetic) are designed to elicit a homogeneous response of physiological activation and subjective feelings of anxiety or frustration. They also allow for a very limited set of behavioral options (e.g., button press, serial subtraction, etc.). Role-play tasks, on the other hand, mimic real-life stressful situations and result in more diverse emotional and complicated behavioral responses. Unlike paradigms which manipulate the cognitive or emotional response to a task (such as the perceived control paradigms used by Sieber et al. (1992) and Weisse, Pato, McAllister, Littman, Breier, Paul, & Baum (1990) and the directed emotion task of Futterman et al. (1994)), realistic role-plays bring subjects' habitual ways of perceiving and responding to stress into the laboratory (Goldstein & Simonson, 1971). This ecological validity may be important for bridging between the patterns of immune changes seen in experimental laboratory tasks and those of descriptive "real-life" stress situations (van Doornen & Turner, 1992). In the present study, we use a confrontational role-play, which brings out responses varying from assertion to capitulation, and examine the psychological, behavioral, physiological, and immune responses to this task compared to a resting control task. This study will, therefore, examine the pattern of immune changes from a more naturalistic stressor which may elicit subjects' typical responses to an interpersonal conflict. A second question is whether the behavioral response of assertion vs capitulation is predictive of differential immune changes to the stressor.

METHODS

Subjects

Twenty male subjects were recruited through advertisements in local university newspapers. All subjects were between the ages of 20 and 40 years (mean = 29.3). Exclusionary criteria in the study, obtained by self-report and questionnaires, included use of prescription drugs, psychiatric diagnosis, any medical condition known to affect immune status, cigarette use, and current abuse of alcohol or nonprescription drugs. Subjects were also eliminated if they reported depressive symptomatology, using a cutoff of 11 points or higher on the Beck Depression Inventory (Beck, Ward, Mendelson, Mock, & Erlbaugh, 1961) or major recent life events such as a death in the immediate family.

Design and Procedures

Each subject came to the laboratory for two 90-min sessions, with each session at least 1 week apart. Both sessions began after an overnight fast, measured by self-report, and were run at the same time (either 8:30 or 10:00 AM) to control for diurnal fluctuations. The two sessions differed in regard to task, with order of the task randomized between subjects. Subjects with symptoms of colds or other minor infections during the week prior to each experimental session were rescheduled. All subjects also refrained from aspirin, nonsteroidal antiinflammatory agents, and antihistamines during the 24 h preceding the test day.

Upon arrival at each laboratory session and completion of the informed consent form on the first session, subjects were seated in a comfortable recliner chair and completed the Profile of Mood States (POMS) (McNair, Lorr, & Droppleman, 1971). A small butterfly needle and intermittent infusion set were secured into an antecubital vein, thus allowing for repeated blood sampling without further invasive procedures. Transducers for heart

rate, skin conductance, and blood pressure were applied. Subjects were instructed to refrain from any movement as much as possible.

Following the setup, a 15-min baseline period was begun. A pretask blood sample was drawn at the end of the baseline period (approximately 30 min after insertion of the butterfly needle). The subject then filled out the Stress Symptom Ratings (SSR) questionnaire (Naliboff et al., 1991). Upon completion of the questionnaire, subjects proceeded with either the role-play task or the control task (viewing a videotaped lecture on a health topic).

The control task was designed to elicit restful alertness. The role-play task is adapted from that used by Moos and Solomon (1965) with rheumatoid arthritics. Subjects are given the following instructions by an examiner:

> I am going to portray a situation that may well be similar to one you have exper enced before. Respond as you would in real life, as if the situation were an actual one. I am the complaint manager of a department store from which you have just purchased a warranted, "top-end" electric razor. The razor is defective or unsatisfactory, and you are returning it for a full refund.

The examiner then begins to play the complaint manager, marking the start of the role-play task. The examiner plays an extremely unreasonable, stubborn, and unpleasant individual, who blames the subject for whatever problem occurs with the defective item. The examiner tells the person that the item is factory pretested and that there have never been other complaints. The examiner includes prompts such as questioning the honesty of the subject and persistently refusing to grant a refund or exchange, and increases hostility and unpleasantness as long as the subject persists. The role-play is terminated when the subject gives up, seeks higher authority, or "leaves" in anger. There is a maximum duration of 15 min allowed. The mean length of the role-play task was 6.3 min (SD = 2.1) with a range of 3.7 to 12.3 min. The control task is set at 12 min.

A second blood sample was drawn at the end of both task periods, at 12 min for the control task and at 4 to 12 min for the role-play task. A second SSR mood questionnaire was also administered following the task for a subjective rating of the task period. The role-play was videotaped for subsequent behavioral ratings of assertion.

Physiological Measures

A PC 386 computer was used for on-line data acquisition and storage. Physiological measures consisted of heart rate (HR), skin conductance level (SCL), and blood pressure (BP). A Grass Model 7D polygraph provided signal conditioning and strip chart recording functions for HR and SCL. HR and SCL were recorded using Sensormedics recessed disk, Ag/AgCl electrodes filled with TECA electrolyte and secured to the skin with double-faced adhesive collars. HR electrodes were attached at the jugular notch and over a low rib. Electrodes for SCL were attached to the volar surface of the first and third fingers of one hand. The R wave from the EKG was detected by a Coulbourn bipolar comparator and triggered by computer software. SCL was sampled by the computer through an analog to digital converter at each heart rate. BP was recorded using a Bard Biomedical Automatic Blood Pressure Monitor, Model ASP 400. At each cuff inflation, the ASP 400 measured diastolic (DBP) and systolic (SBP) blood pressure using an oscillometric methodology.

Samples of HR and SCL were taken during the last 2 min of every 5 min of the baseline (three, 2-min samples) and control task (two, 2-min samples). Since the length of the role-play was open ended and averaged 6 min, one 2-min sample was taken from minute 3 to minute 5. Blood pressures were obtained at 5-min intervals during baseline, immediately following the 2-min acquisition of HR and SCL, excluding the last 5 min of

baseline prior to the drawing of the baseline blood sample. For the control task, blood pressure was taken at 5-min intervals (two samples), and for the role-play, blood pressure was taken after the first 5 min and again at the end of role-play task. Only the blood pressures taken at the end of the control and role-play tasks were used in the analysis to be comparable with the timing of the blood draws.

Psychological Measures

Two psychological measures were used in this study, one to measure the subjective evaluation of the experimental conditions and the other to assess general mood as a potential predictor of the response to stress. The SSR scale was administered pre- and posttask to track the acute affective change during the task period. This questionnaire consists of a series of 12 visual analog scales anchored by mood-related adjectives and grouped into six subscales: Arousal, Stress, Anxiety, Anger, Fatigue, and Attention. The SSR has been shown to be responsive to the subjective stress associated with mental arithmetic (Naliboff et al., 1991). The POMS is a well-standardized measure comprising six scales (Tension–Anxiety, Depression–Dejection, Anger–Hostility, Vigor–Activity, Fatigue–Inertia, Confusion–Bewilderment), which assesses general mood and psychological state (McNair et al., 1971).

Behavioral Ratings

Two raters with backgrounds in behavioral science independently rated each videotaped role-play for assertiveness. Raters were given training and practice sessions using nonstudy subjects to achieve reliability. The verbal and nonverbal behaviors used to operationalize assertion were derived from two major criteria: 1) being insistent and not giving up and, 2) keeping on task (Rimm & Masters, 1974). As an example for the first criteria, highly assertive behavior would include demanding to seek some form of higher authority after a lack of results with the complaint manager. A low assertion subject would give up when told no higher authority existed. Highly assertive behavior by the second criteria required subjects to stay focused on their goal of a refund or replacement and not be led off-track by the agenda of the complaint manager. For example, low assertive behavior might be to argue about the subject's own qualifications to use a razor (prompted by the complaint manager) instead of continually returning to the point that the razor is defective and is the store's responsibility. Based on the above criteria, each subject was given a global measure of assertiveness for the entire role-play using a 10-point scale (1 = minimal to 10 = maximal). Raters were blind as to experimental hypotheses or key questions. Interrater reliability for the global assertiveness measure was 0.80 and the range for behavioral ratings of assertion was 2.5 to 10.

Immune Measures

Measures of immune system function were obtained from the blood drawn before and immediately after the task period. These included counts of specific lymphocyte subtypes and an *in vitro* assay for NK cell cytotoxicity. For the assessment of cell numbers, lymphocytes were isolated from blood by centrifugation over Ficoll–Paque (Pharmacia, Piscataway, NJ), followed by removal of adherent cells by plastic adherence for 1 h. Absolute counts (per mm^3) and percentages of six T cell subtypes and total B cells were assessed using two color flow cytometry (Fahey, Prince, & Weaver, 1984). The identifying monoclonal antibody and the corresponding cell subtype were as follows; Leu 2 (suppressor/cytotoxic, CD8); Leu 3 (helper/inducer, CD4); Leu 4 (total T cells, CD3); Leu 7 (large granular lymphocytes that are the phenotype of cytotoxic cells, CD57); Leu 11 (Fc

receptors; most are NK, CD16); Leu 19 (mostly NK cells, CD56); and Leu 16 (total B cells, CD20). Percentages of $CD8^+$ cells with Leu 7 receptors ($CD8^+CD57^+$) were also assessed.

To assess NK cell cytotoxicity, lymphocytes were isolated using Lympho Prep (Accurate Antibodies, Westbury, NY) by buoyant density centrifugation. Mononuclear cells were washed three times and resuspended at 2.5×10^6 cells/ml in RPMI 1640 with 10% heat-inactivated fetal calf serum. Isolated cells were 95% viable by tryptan blue exclusion and Wright/Giemsa tests. NK cell cytotoxicity was measured using ^{51}Cr-labeled K562 target cells (Romano, 1986). Target cells were labeled with 100 μCi of ^{51}Cr for 1 h at 37°C, washed three times in complete medium, and resuspended. Effector and target cells were incubated for 4 h in V-bottom microplates. Each test well contained 100 μl of 1×10^5/ml ^{51}Cr-labeled K562 target cells and 100 μl of effector cells (2.5×10^5/ml or 1×10^5/ml) to produce final effector-to-target (E/T) ratios of 25/1 and 10/1, in triplicate. Wells with ^{51}Cr-labeled K562 in medium alone or with 0.1 ml *N* HCl were used to assess spontaneous and maximum release respectively. After 4-h incubation, 100 μl of supernatant was placed in a gamma counter to assess extent of ^{51}Cr release. Percentage of NK cell cytotoxicity (percentage lysis) was calculated as [experimental(cpm)-spontaneous cpm/total(cpm)-spontaneous (cpm)]. Following the procedures of Benschop et al. (1994b), Schedlowski et al. (1993), and Pike, Smith, Hauger, Nicassio, and Irwin, (1995), we also calculated for each sample an adjusted NK cytotoxicity, based on the percentage of NK cells in the effector mix. Each percentage lysis was first transformed into a value corresponding to number of 10^6 effector cells needed to kill 20% of 10^5 target cells, and then divided by the percentage of NK cells to total lymphocytes.

STATISTICAL ANALYSIS

The major hypotheses of stress-related changes were examined by comparing measures following the role-play with those following the control (video) task. Analyses of the major psychological, physiological, and immunological variables were performed using analysis of covariance (ANCOVA) with each posttask measure as the dependent variable and the pretask baseline for that day as the covariate (Dixon, 1992). The within-subject statistical comparisons between the two tasks (video and role-play) were therefore performed on values adjusted for variability in baseline measures across days. This statistical approach (analagous to that of "residualized scores") is preferable to simple change scores because it takes into account the correlation between baseline and posttask values common in laboratory studies (for a discussion see Manuck, Kasprowicz, & Muldoon, 1990). ANCOVA is particularly appropriate if conditions are randomly assigned (in this case order of tasks were randomly determined for each subject; Cohen & Cohen, 1975). The association between changes in NK cell numbers and NKCC and the behavioral ratings of assertion, and between NK measures and physiological arousal were examined using correlation coefficients. Alpha was set at $p < .05$ throughout.

RESULTS

Subjective Ratings

Subjective ratings, using the SSR, were made each session before and immediately following the task (see Table 1). Significant task effects were found for the ANCOVAs (with prestress values as covariates) for Arousal [$F(1,16) = 21.37$, $p < .001$], Stress [$F(1,16) = 40.92$, $p < .001$], Anxiety [$F(1,16) = 22.92$, $p < .001$], Anger [$F(1,16) = 44.07$, $p < .001$], Fatigue [$F(1,16) = 29.42$, $p < .001$], and Attention [$F(1,16) = 10.88$, $p < .01$].

TABLE 1
Effect of Role-Play on Psychological Measures

Variable	Role-play		Video	
	Pretask	Task	Pretask	Task
Psychology				
Arousal	4.15 ± 2.1	7.82 ± 1.4	4.21 ± 1.9	5.16 ± 2.4
Stress	1.73 ± 1.5	6.14 ± 1.9	1.77 ± 1.3	2.74 ± 2.0
Anxiety	1.58 ± 1.3	4.90 ± 1.6	1.51 ± 1.2	2.64 ± 1.8
Anger	1.88 ± 1.2	7.19 ± 2.1	1.88 ± 1.6	3.69 ± 2.1
Fatigue	4.18 ± 1.7	7.22 ± 1.3	4.36 ± 1.4	4.53 ± 1.4
Attention	6.59 ± 2.3	7.80 ± 1.7	6.59 ± 1.8	5.94 ± 2.4

Note. Values are average distance (±SD) on a 10-cm visual analog scale.

In all cases the role-play task led to greater stressful mood ratings compared to the video task (including increased energy/decreased fatigue).

Physiological Measures

The physiological measures were analyzed in a manner analogous to the subjective ratings (see Table 2). Significant task effects were found for HR [$F(1,18) = 12.5$, $p < .01$], SBP [$F(1,17) = 23.84$, $p < .001$], and DBP [$F(1,17) = 70.15$, $p < .001$], with greater values for these measures following the role-play task compared to the video task. Overall then, the subjective and physiological measures confirm that the role-play task led to significant arousal compared to the video condition.

Absolute Numbers of the Cell Subtypes

Absolute numbers of the various lymphocyte subtypes were assessed from blood drawn before the task period and at the end of the task. The posttask samples were analyzed using ANCOVA with the daily pretask baseline values as covariates. Means and standard deviations for these measures are contained in Table 3. There were significant task effects for CD8 suppressor/cytotoxic T cells [$F(1,17) = 5.09$, $p < .05$], CD4 helper/inducer T cells [$F(1,16) = 6.70$, $p < .05$], CD20 B cells [$F(1,16) = 11.00$, $p < .01$], and for phenotypes associated with NK cells, i.e., CD56 [$F(1,14) = 16.27$, $p < .01$], CD16 [$F(1,16) = 16.28$, $p < .001$], and CD57 [$F(1,17) = 6.98$, $p < .05$]. As shown in Table 3, CD8 and NK cells increased following the role-play relative to the video task, while CD4 and CD20 subsets decreased.

TABLE 2
Effect of Role-Play on Physiological Measures

Variable	Role-play		Video	
	Pretask	Task	Pretask	Task
Physiology				
Heart rate (bpm)	60.05 ± 11.0	66.86 ± 12.0	59.14 ± 7.9	61.48 ± 7.8
Systolic BP (mm Hg)	121.24 ± 7.8	133.50 ± 10.76	121.47 ± 8.4	119.63 ± 10.8
Diastolic BP (mm Hg)	69.29 ± 5.0	79.53 ± 5.3	70.13 ± 5.1	70.21 ± 5.2
Skin conductance (μSiemens)	12.59 ± 10.3	17.05 ± 9.7	9.35 ± 5.4	13.38 ± 7.8

Note. Values are ±SD.

TABLE 3
Effect of Role-Play on Absolute Counts (per mm^3) for Cell Subsets

	Role-play		Video	
Variable	Pretask	Task	Pretask	Task
CD8	612.11 ± 238.5	692.68 ± 142.0	604.21 ± 173.73	640.37 ± 183.1
CD4	856.67 ± 253.8	816.06 ± 261.0	841.28 ± 286.3	856.72 ± 255.7
CD3	1390.65 ± 387.6	1375.83 ± 388.4	1383.94 ± 407.2	1408.59 ± 377.1
CD20	231.72 ± 110.6	208.33 ± 90.1	257.94 ± 150.3	253.17 ± 122.5
CD57	233.95 ± 273.3	306.95 ± 192.7	192.89 ± 186 1	223.11 ± 216.0
CD16	209.00 ± 72.5	325.39 ± 114.5	190.00 ± 59.1	208.8 ± 60.6
CD56	282.50 ± 89.4	403.56 ± 121.0	252.63 ± 64.6	274.69 ± 58.8
CD8+CD57+[a]	22.85 ± 16.5	27.95 ± 15.7	21.40 ± 15.9	23.80 ± 17.9

Note. Values are average counts ±SD.
[a] Percentage of CD8 cells with Leu 7 receptors.

NK Cytotoxicity

NK cytotoxicity was analyzed in the same manner as the cell subtypes. For percentage lysis, no effect of task as found for either concentration (see Table 4). However, the *adjusted* NK cytotoxicity (adjusted for percentage of NK cells in the effector mix) did show significant task effects for both concentrations (for 10/1, $F(1,15) = 7.71$, $p < .05$; for 25/1 $F(1,15) = 7.77$, $p < .05$). In both cases, adjusted NK cytotoxicity was decreased following the role-play relative to the video task.

Relationships among Measures

Based on a priori hypotheses, correlations between several of behavioral, physiological, and immunological variables were performed for the role-play task. All correlations involving change were performed using residualized scores (posttask values adjusted for baseline values). Behavioral ratings of assertion during the role-play did not correlate significantly with change in NKCC (percentage lysis or adjusted NK cytotoxicity), number of NK cells (CD16), or the other immune or physiological measures. Correlations between assertion and change in subjective stress (assessed by the SSR scales) were positive but not significant (range 0.25 to 0.37). There were significant correlations between changes in NK cell numbers during the role-play and changes in SBP ($r = .55$, $p < .05$) and HR ($r = .50$, $p < .05$), and a smaller nonsignificant correlation with changes in DBP ($r = .37$). The initial POMS scales were not significantly related to subjective,

TABLE 4
Effect of Role-Play on NK Cell Cytotoxicity

	Role-play		Video	
Variable	Pretask	Task	Pretask	Task
		NK cytotoxicity		
(25/1)	26.13	32.85	27.05	32.65
(10/1)	12.41	16.27	13.14	15.83
		Adjusted NK cytotoxicity (per cell)		
(25/1)	52.26	45.74	63.35	75.84
(10/1)	61.44	52.18	76.03	86.29

Note. NK cell cytotoxicity measured in percentage lysis. Adjusted NK cell cytotoxicity is adjusted for percentage of NK cells to total lymphocytes in effector mix (±SD).

physiological, or immunological change during the task. Role-play length was not significantly correlated with change in any of the subjective, immune, or physiological measures although there was a trend toward a significant relationship between task length and change in NK cell numbers ($r = .44$, $p = .06$). Since none of the other immune, subjective, or physiological measures were related to task length this correlation is unlikely to reflect differences in general arousal or response to the task, but may instead result from there being a rapid peak in the time course of increases in NK cell numbers following onset of the stress.

DISCUSSION

The brief confrontational role-play used in this study led to significant subjective and physiological arousal. Compared to the video watching task, the role-play resulted in significantly increased HR, blood pressure, and subjective ratings of anxiety, anger, and stress. There was also an increase in circulating NK (CD16, CD56, and CD57) cells and suppressor/cytotoxic T cells (CD8). T helper (CD4) and B (CD20) cells significantly decreased following the role-play compared to the video. These results are consistent with a variety of studies using brief, arousal generating tasks and demonstrate the rapidity of immune changes to psychological challenge. There were also significant relationships between stress-related increases in the cardiovascular measures and the numbers of circulating NK cells. This finding lends further support to the hypothesis of sympathetic nervous system activation as a primary mechanism for increases in NK cell numbers under challenge. The behavioral ratings of assertion did not correlate significantly with changes in either NK cell numbers or NKCC. The length of the role-play for the current study was variable (and for some subjects very brief) and determined by the subject's behavior. While role-play length was not significantly associated with the various dependent variables, it is possible that the variability and length of the task may have limited the sensitivity of this particular task for detecting the impact of behavioral individual differences on immune system change.

Compared to examination or game stressors a role-play task might lead to a different pattern of immune system changes because of the quality of emotion generated and the "real-life" nature of the response options. For example, we can compare the current results to those from another brief stressful task used in our laboratory, pressured mental arithmetic (MA) (Naliboff et al., 1991, 1995). In the mental arithmetic task, subjects are asked to perform serial subtractions starting at 3500. A white-coated "examiner" administers the test using a professional and serious demeanor and verbal prompts designed to indicate the subject is not performing as well as expected. Data from a recent study using this task are especially good for comparison since the subject population, control condition (video watching), subjective measures, and physiological measures are identical (Naliboff et al., 1995). In addition the MA study used the same immune measures as the present study, and assays for the two studies were performed in the same laboratories using identical procedures. Both laboratory tasks led to similar cardiovascular arousal ($\cong$8 bpm for heart rate, $\cong$13 mm Hg for systolic blood pressure). Both tasks also resulted in increased ratings for all of the measures of subjective stress. Inspection of the relative changes across the two tasks for the individual SSR dimensions indicate similar increases in stress and anxiety for the two tasks but perhaps greater anger ratings for the role-play compared to MA (an increase of 5.0/10 vs 3.3/10).

Both the role-play and MA tasks led to increases in CD8, CD57, CD16, and CD56 cell numbers compared to a control task. The changes in immune measures were similar across the studies with the exception of NKCC. Mental arithmetic led to increases in NKCC but

this difference could be completely accounted for by increases in the proportion of NK cells in the effector mix. Thus, adjusted NK cell cytotoxicity did not increase during the mental arithmetic stressor. This finding is consistent with data from other brief laboratory stress tasks (Bachen, Manuck, Cohen, Muldoon, Raible, Herbert, & Rabin, in press; Brosschot et al., 1992), although some studies have reported increased "per cell" NK cytotoxicity especially under conditions of intense stress (Schedlowski et al., 1993). The role-play task, however, led to significantly *decreased* adjusted NK cell cytotoxicity. Previously, decreased NK cell cytotoxicity has only been associated with chronic human stressors and acute stress in animals (Cunnick et al., 1988; Glaser et al., 1986; Herbert & Cohen, 1993; Irwin, Daniels, Risch, Bloom, & Weiner, 1988; Kiecolt-Glaser et al., 1986; Lewis et al., 1986; Morrow-Tesch et al., 1993; Schlesinger & Yodfat, 1991; Zalcman et al., 1991). If replicated, the current results indicate that NK cytotoxicity may be particularly affected by qualitative aspects of a stressor. Further research examining this hypothesis may lead to a better understanding of the often divergent findings from chronic and acute stress models. Clearly, more data is also needed on the question of the most appropriate NK cytotoxicity measure and the interpretation of various adjusted "per-NK-cell" cytotoxicity values.

The differences in NK cytotoxicity changes across various activating experimental tasks suggest the potential importance of dimensions of the behavioral and emotional response to challenge in addition to that of autonomic arousal. For example, a task may be perceived as positive vs negative or require active vs passive responses, or lead to feelings of control vs helplessness. It may be that these other dimensions interact with arousal so that no significant physiological responses will occur without autonomic activation, but the pattern of neuroendocrine and immune responses under arousal conditions may vary according to the emotional and behavioral specifics of the situation.

There are currently no immune data from experiments which have directly examined qualitative aspects of stressors such as those mentioned above under conditions of similar arousal. However, there is supporting evidence for the role of nonarousal-related factors from laboratory studies which have examined stressor and subject characteristics. Sieber et al. (1992) found that perceived stress controllability that was manipulated by increased failure did not alter the pattern of immune responses. However, a unique condition involving a total lack of response options coupled with loud tones led to decreased NKCC, a finding similar to that of the present study. Unfortunately, the investigators did not assess physiological measures of arousal, so the activation from the various conditions cannot be compared. Chronic stress may also alter the immune response to acute stress. Pike et al. (1995) found decreased NKCC for chronically stressed subjects and increased NKCC for controls following MA, even though both groups showed the typical increase in NK cell numbers during the stress. It is unclear if these differences are related to differences in arousal or other factors. We found that NKCC increased for younger subjects but was unchanged for older subjects following mental arithmetic, even though all other immune (including NK numbers) and physiological measures were similar (Naliboff et al., 1991). Finally, Futterman et al. (1994) using direct role-play of emotions by method actors, found some immune variables (CD8, CD16, CD56, NKCC) were related to activation (assessed by HR and body movement) but proliferation to the mitogen PHA was more directly related to emotional content (positive or negative).

Although a variety of descriptive studies have examined the relationship between psychological characteristics and basal immune measures, laboratory studies offer an alternative and complementary approach. If the time frame of the hypothesized immunological changes is appropriate for the experimental setting, laboratory studies allow for

clearer tests of causation and mechanisms of psychological–immunological interactions. The development of laboratory paradigms which can differentiate responses related to changes in arousal, quality of emotion, or behavior might be an important step in determining how these aspects of challenge effect immunity and potentially the mechanisms involved (including neuroendocrine mediators). Laboratory tasks which mimic real-world stress and result in complex behavioral responses may also serve as better probes for the effects of stable individual difference variables on stress–immune relationships. Future tasks, for example, could be designed to elicit homogeneous responses on a general stress measure (e.g., arousal) but heterogeneous responses on other dimensions (e.g., positive/negative valence or propensity to respond). Use of behavioral ratings in addition to self-report of emotion would be an important additional tool for these studies. In this way, laboratory experiments might better be able to test hypotheses drawn from descriptive studies of personality measures or high risk groups.

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