

Reactivity and Vulnerability to Stress-Associated Risk for Upper Respiratory Illness

SHELDON COHEN, PhD, NATALIE HAMRICK, MS, MARIO S. RODRIGUEZ, PhD, PAMELA J. FELDMAN, PhD, BRUCE S. RABIN, MD, AND STEPHEN B. MANUCK, PhD

Objective: We tested the hypothesis that the greater a person's laboratory stress-elicited elevation in cortisol, the greater the life stress-related risk for upper respiratory infection (URI). We also tested the prediction that the greater the laboratory stress-elicited rise in natural killer cell (NK) cytotoxicity, the smaller the life stress-related URI risk. Finally, we explored whether sympathetic nervous system (SNS) and enumerative immune reactivities to laboratory stress moderate the relation between life stress and URI. **Methods:** At baseline, 115 healthy subjects were administered a negative stressful life events checklist and were tested to assess their SNS (blood pressure, heart rate, and catecholamines), HPA (cortisol), and immune (NK cell cytotoxicity and lymphocyte subsets) reactivities to laboratory speech tasks administered 2 weeks apart. Responses were averaged across the two laboratory assessments to create reactivity scores. After these assessments were completed, participants were followed weekly for 12 consecutive weeks. At each follow-up they completed a measure of perceived stress experienced over the last week. They were also instructed to contact the study coordinator if they had a cold or flu at any time during follow-up. A health care worker verified reported illnesses. **Results:** In a traditional prospective analysis, high cortisol reactors with high levels of life events had a greater incidence of verified URI than did high reactors with low levels of life events and low reactors irrespective of their life event scores. Using hierarchical linear modeling, CD8⁺ number, Natural Killer (NK) cell number, and NK cell cytotoxicity, each interacted with weekly perceived stress levels in predicting concurrent occurrences of self-reported URIs. For these outcomes, low immune reactors were more likely to experience an URI during high stress than low stress weeks. High immune reactors did not exhibit differences in weekly URIs as a function of weekly stress level. The SNS reactivity markers did not moderate the association of stress and URI incidence in either analysis. **Conclusions:** Acute HPA and immune responses to laboratory stressors are markers of how vulnerable people are to the increased risk for URI associated with stressors in the natural environment. **Key words:** reactivity, cardiovascular, immune, cortisol, upper respiratory illness, common cold.

BHD = behavioral-health diary; DBP = diastolic blood pressure; HLM = hierarchical linear modeling; HPA = hypothalamic-pituitary-adrenal cortical axis; HR = heart rate; PSS = Perceived Stress Scale; SNS = sympathetic nervous system; SBP = systolic blood pressure; URI = upper respiratory infection.

INTRODUCTION

Although psychological stress has been found to be associated with greater incidence of URI, only a fraction of those with high stress develop illness (eg, Refs 1-4). Reactivity, the biological changes displayed by individuals in reaction to acute laboratory stressors, may be an important factor for explaining variability in stress-induced susceptibility to URI (5-7). The under-

lying assumption here is that the way people respond to stressors in the laboratory reflects the pattern and magnitude of biological responses they exhibit when confronted with stressors in naturalistic settings. Because the stress-induced responses of the SNS, HPA, and immune system are thought to modulate host resistance to infectious agents, knowing the propensity of persons to respond in a specific way in the laboratory should increase our ability to predict if they will respond to naturalistic stressors with increased or decreased susceptibility.

There is growing evidence for stable individual differences in biological response to acute stressors. The human reactivity literature has primarily focused on the response of the sympathetic nervous system. In this work, acute laboratory stressors generally elicit rises in epinephrine, norepinephrine, blood pressure, and heart rate (8). These responses have been found to be reliable across both time and task and hence represent a stable trait of the individual (9-11).

Another commonly studied response to stressor exposure is an increase in the levels of the steroid hormone cortisol that is released by the adrenal cortex. The magnitude of acute-stressor-induced cortisol response is also relatively stable over time (9, 12). Cortisol response to acute stressors is slower than SNS response (13) and correlates only moderately with SNS response (9). Because cortisol can suppress the inflammatory immune response, the release of more cortisol under stress could be associated with greater susceptibility to infectious agents (14, 15).

From the Department of Psychology (S.C., N.H.), Carnegie Mellon University, Pittsburgh, PA; Research and Development (M.S.R.), Psychological Assessment Resources, Inc., Lutz, FL; Department of Epidemiology and Public Health (P.J.F.), University College London Medical School, London, UK; Departments of Pathology and Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA (B.S.R.); and Department of Psychology (S.B.M.), University of Pittsburgh, Pittsburgh, PA.

Address reprint requests to: Sheldon Cohen, PhD, Carnegie Mellon University, Department of Psychology, 5000 Forbes Ave., Pittsburgh, PA 15213-3890. Email: scohen@andrew.cmu.edu

Received for publication March 13, 2001; revision received July 25, 2001.

REACTIVITY AND URI

Acute laboratory stressors have also been found to alter immune response including numbers of key populations of lymphocytes in circulation (16, 17) as well as functional aspects of immunity, including mitogen-stimulated lymphocyte proliferation (16–20) and NK cell cytotoxicity (9, 18). Most of these responses have also been found to be stable across time (9, 19, 21). These responses include those that one would expect to increase (eg, stress-induced increases in NK cytotoxicity) and decrease (eg, stress-induced decreased in mitogen-stimulated lymphocyte proliferation) host resistance to infectious agents.

In the only direct tests of reactivity as a moderator of the association between stressful life events and respiratory disease, Boyce and colleagues (5, 6) found that both cardiovascular and immune reactivity moderated children's responses to naturalistic stressful events. Although their results are complex, there is the suggestion that high blood pressure reactors are at greater risk for illness under high stress. There also is the suggestion that reactivity as assessed by some immune responses (CD4⁺/CD8⁻ cells and increases in pokeweed-stimulated lymphocyte proliferation) was associated with increased rates of URI under high stress.

In the current study, we sought to expand our understanding of the role of biological reactivities as vulnerability factors in the association between psychological stress and infectious illness. The study was designed to test three hypotheses.

1. Because immune competence in the face of infectious agents may be compromised by cortisol (14, 15, 22), we expected that those with larger acute stress-induced increases in cortisol would be more likely than their less reactive counterparts to have increased risk for URI when naturalistic levels of stress were high vs. low. In contrast, we expected that people with smaller cortisol reactivity would show little to no stress-induced increase in URI incidence.

2. Because NK cells play a role in killing invading viruses, we expected that those with a smaller acute stress-induced rise in NK cytotoxic reactivity would be more likely to have an episode of URI when naturalistic levels of stress were high vs. low, whereas those with greater increases in NK cytotoxicity would be less affected or possibly unaffected by stress. Because there is little evidence establishing associations between numbers of immune cells in circulation and incidence of URI, we made no predictions regarding enumerative immune reactivity.

3. SNS-mediated reactivities have been found to be associated with immune outcomes that both increase (18, 23) and decrease host-resistance (18). Therefore, predictions regarding the associations of these reactivities were difficult to make. However, we sought to

replicate the previous findings of Boyce and colleagues (5, 6), which suggests that greater acute stress-induced rises in blood pressure are associated with higher rates of illness in those confronted with stressful events in their natural environment.

We incorporated a number of methodological approaches not usually used in this literature. First, we simultaneously assessed SNS, HPA, and immune reactivity. Second, we conducted each of the reactivity assessments *twice* and aggregated across the two laboratory sessions to create more reliable scores than the "one-shot" assessments often used. Third, the URI outcome was not determined on the basis of symptom levels alone, but on nurse practitioner verification and participant self-report of illness. In addition, our analyses controlled for the effects of neuroticism, a personality characteristic associated with a bias to report more stress, symptoms, and illness (24, 25).

We also expanded on previous work by analyzing the data in two ways. The first approach was a traditional prospective analysis in which we addressed whether between-subject differences in background/chronic stress levels and reactivity assessed at baseline predict subsequent URI incidence. In the second approach, we prospectively looked at reactivity measures as predictors of which subjects would subsequently respond to rises in their own weekly perceived stress levels with increased risk for episodes of URI during these high stress weeks.

METHODS

Participants

Participants were solicited via electronic bulletin boards, school newspaper, and word of mouth. They were eligible to participate if they were age 18 to 30 years, were students of either the University of Pittsburgh or Carnegie Mellon University, had no infectious illness 2 weeks before each session, had no chronic illness, no personal history of cancer, no autoimmune disorders, no current or history of psychological disorder, consumed no more than 12 alcoholic beverages on average per week, did not use street drugs, and were not currently pregnant or lactating. One hundred fifty-one people met these criteria. Of the potential participants, 115 (71%) participated. The main reason for nonparticipation was missing scheduled appointments (85%); the remaining nonparticipants (15%) had difficulty with catheterization. Four cohorts of participants were run (Fall 1996, $N = 13$; Spring 1997, $N = 44$; Fall 1997, $N = 28$; Spring 1998, $N = 30$). The overall sample had a mean age of 21.1 years ($SD = 2.7$ years) and was 47% men, 53% women; 92% single; 76% white, 10% African American, 7% Asian, 2% Hispanic, and 5% "other race." Participants received a total of \$120 for participation in the two sessions and the 12-week follow-up.

Procedures

All participants attended two laboratory sessions for the collection of reactivity data. After these assessments were completed,

participants were followed weekly for 12 consecutive weeks. At each follow-up, they completed a diary that asked question about their levels of stress and their health status during the preceding week.

The laboratory sessions lasted approximately 2 hours and were scheduled exactly 2 weeks apart at the same hour of the day (either 7:00 AM or 9:30 AM) to control for diurnal variation. Experimental procedures at the two sessions were nearly identical. Participants were asked to abstain from tobacco products, vigorous exercise, caffeine, and food or beverages (except water) for 8 hours before sessions, and to abstain from over-the-counter medication for 24 hours before sessions. Participants were seated upright in a comfortable chair. Each session consisted of catheterization, followed by a 30-minute rest period, followed by a simulated public-speaking task. Baseline cardiovascular measures were taken 25, 27, and 29 minutes into the rest period (and averaged), and baseline catecholamine and immune measures were taken immediately after the rest period. Baseline cortisol were assessed at home (as recommended by Kirschbaum (24)) during the week in between the two laboratory sessions. The collection occurred on the same day of the week as the lab sessions and at the same number of hours after awakening as when the stress sample was taken in the laboratory (approximately 3 hours, although it varied between participants).

Each speech task consisted of a 2-minute preparation for a speech in which the participants defended themselves against an alleged transgression (shoplifting or traffic violation), followed by 3 minutes of videotaped speech delivery. Sympathetic nervous system and immune measures were collected during speech delivery. Specifically, SBP, DBP, and HR were collected at 1 and 2.5 minutes into the speech (and averaged), and epinephrine, norepinephrine, NK cytotoxicity, and lymphocyte subsets were collected at 2 minutes into the speech. HPA axis (cortisol) response was assessed 17 minutes after the completion (22 minutes after onset) of the speech task. Residualized change scores, the residual from the regression predicting task scores from baseline scores, were used to represent the reactivity scores for each visit.

One of the assumptions of the reactivity hypothesis is that reactivity represents a stable manner in which an individual responds to acute stressors (7). In a previous publication (9), we reported the test-retest correlations between residualized change scores for each outcome across the two occasions of testing in this sample. Reactivities considered to be stable (ie, exhibiting moderate to high test-retest correlations) were: systolic blood pressure, diastolic blood pressure, heart rate, cortisol, norepinephrine, CD8⁺ cell number, NK cell number, and NK cytotoxicity (test-retest correlations greater than 0.40, *p* values < .05). Each reactivity index displaying such stability was then averaged across sessions to create the reactivity score for that measure. Session-averaged reactivity scores were used for analyses in the current report. Note that each of the reactivity indices found to be unstable was discarded from the analyses reported here. Because we were interested in attempting to replicate the findings of Boyce and his colleagues (5, 6), we also examined the stability of two other measures used by them but not considered in our earlier work: the CD4⁺/CD8⁺ ratio, and mean arterial blood pressure [DBP + 1/3(SBP - DBP)]. Both had acceptable test-retest correlations (0.39 and 0.64, respectively; *p* < .001).

Also, before beginning the rest period for each session, participants completed various questionnaires. Specifically, negative major life events experienced over the past 12 months and demographic information were collected during the first laboratory session. We collected information concerning neuroticism levels during the second laboratory session.

After completion of both laboratory sessions, participants were instructed on how to complete the weekly behavioral-health diary.

BHDs were mailed electronically to the participants to be completed and returned on the same day of the week as their laboratory visits. Although the majority of participants had electronic mail accounts, those who did not (less than 5%) were phoned on the appropriate day to collect the BHD information. Participants who did not respond on the appropriate day were immediately contacted and interviewed or reminded to submit their electronic BHD. Specifically, participants provided information concerning perceived stress levels and whether they believed themselves to have had a cold or flu since completion of the last diary. They were also asked to notify the study coordinator if they developed a cold or flu between assessments. Those reporting an URI (either at assessment or between assessments) were immediately scheduled for an appointment at the student health center where they were examined by a nurse practitioner for verification of an URI. One participant was delinquent in returning the BHDs and was terminated from the follow-up period, resulting in *N* = 114. Of the remaining participants, the majority (87%) completed and returned all 12 BHDs. Of the 1368 potential weekly diaries (114 participants × 12 weeks), 1343 (98%) complete diaries were received.

Measures—Reactivity Sessions

Cardiovascular outcomes. An occluding cuff was placed on the participant's left arm for automated measurement of heart rate and blood pressure using either a Dinamap XL Vital Signs Monitor or Citikron Dinamap.

Immune assays. Enumerative assays were assessed in whole blood using dual color fluorescence analysis with a Becton Dickinson FACScan flow cytometer (San Jose, CA). Lymphocyte subsets were analyzed using monoclonal antibodies labeled with either fluorescein or phycoerythrin to quantify CD3⁺ (total T), CD3⁺CD4⁺ (T helper), CD3⁺CD8⁺ (T-suppressor/cytotoxic), CD3⁺CD19⁺ (B), and CD3⁺CD16⁺CD56⁺ (NK) cells. Absolute numbers of cells were calculated from a complete blood count. Pretask and task blood samples were assayed in the same batch on each occasion of testing.

A whole blood ⁵¹Cr-release assay (26) was used to determine percent cytotoxicity to the NK-sensitive erythroleukemic K562 cell line. Pretask and task blood samples were assayed in the same batch on each occasion of testing.

Cortisol assay. Saliva samples collected via salivettes were centrifuged at 3000 rpm for 5 minutes and a 1-ml sample was obtained. Levels of salivary cortisol were determined via time-resolved immunoassay with fluorometric end point detection (DELFI) and are expressed in units of nmol/L (27). Cortisol data were collected for the last 58 participants only.

Catecholamine assays. Blood samples were anticoagulated with EDTA, chilled, and centrifuged; plasma was then removed and frozen at -70°C until analysis. High performance liquid chromatography determinations of epinephrine and norepinephrine, after extraction with alumina, were conducted using a Phase II, reverse phase, 3-μ column. Peak catechol heights were measured automatically by Chromatochart-PC (BAS/IMI) and are expressed in pg/ml. Catecholamine data were collected for the last 58 participants only.

Measures—Background Variables

Negative life events. The stressful life events scale consisted of major events (eg, death of loved one, close relationship breakup) that might happen in the life of the respondent (41 items) or close others (26 items). The events were a subset of those appearing in the List of Recent Experiences (28) and were chosen because of their potential for negative impact and their relatively high frequency of occurrence in population studies. This particular subset has been used in pre-

REACTIVITY AND URI

vious work from our laboratory (1). Participants examined this list of stressors and indicated whether they had experienced a particular event in the past 12 months. For events that were reported to have occurred, participants also provided ratings of the experience as having a positive or negative impact on their lives. A few items, such as death of a family member or emergency hospitalization of a loved one, were assumed to be consensually negative, and the respondent was not asked to provide an impact rating. A negative life events score was derived for each participant by adding the number of events he or she reported that were negative (either consensual or participant rated). To achieve a normal distribution for this variable, we added one to each participant's score before performing a \log_{10} transformation of it.

Neuroticism. Neuroticism has been associated directly with reports of stress levels and reports of symptoms without a physiological basis (24, 25). Because we relied on participant reports of stress levels and participant judgment to determine when they were sick, we statistically controlled for the effect of neuroticism on reported URI occurrence. Neuroticism was assessed during the second laboratory session via the 10-item neuroticism scale from the Eysenck Personality Questionnaire (29). Responses were summed, yielding a neuroticism score ranging from 0 to 10. This scale was found to be moderately reliable ($\alpha = 0.67$).

Standard demographic control variables. Age was scored as a continuous variable. Sex was scored as a dichotomous variable. Race was categorized as being white, African American, or other.

Measures—Weekly Diary

Perceived stress. During each of the 12 weeks of the follow-up period, participants completed the 10-item PSS (30). At each point, participants were asked about stress over the past week. Items on the PSS measure the degree to which, in the past week, participants felt their lives were unpredictable, uncontrollable, and overwhelming (0 = never, 4 = very often). Perceived stress scores for each week were obtained by reversing the scores on the four positive items, then summing across all 10 items. The scale was reliable for all 12 weeks (α values range = 0.87–0.91).

Verified URI. During the 12-week follow-up period, participants were asked to immediately contact the study coordinator if they felt they had developed a cold or flu. The coordinator then scheduled an appointment for an examination by the nurse practitioner. Students were also sent for an examination if at the weekly interview they reported having a cold or flu. The nurse took a temperature reading and examined the participant's eyes; ears; nose; throat; cervical, submandibular, and axillary lymph nodes; and chest/lungs for the presence of abnormalities. Based on the information gathered above, the nurse determined if the participant's signs and symptoms were most consistent with an URI, some other disease process, or no apparent illness. Of the 51 people meeting criteria for verification, 30 actually went to the clinic. Reasons for students not coming in to have their URIs verified included: their URIs occurred over the weekend when the student health center was closed, they failed to contact the study coordinator when sick (but reported having had the URI at the next interview), or they missed their student health center appointments and were better by the time an appointment was rescheduled. Of the 30 participants coming in for verification, 29 were diagnosed as having an URI. In our analyses, only these 29 participants are identified as having a "verified" illness.

Self-reported URI. Participants indicated "yes, no, or unsure" as to whether they had a URI during the last week for each of the 12 weeks. A participant was considered to have a self-reported URI during a given week if he or she answered "yes." They were also considered to have a self-reported URI if they answered "unsure"

and had their URI episode verified by the nurse practitioner. Ninety-three self-reported URI episodes were experienced by 54 of the participants.

Data Analysis

We used multiple logistic regression to examine whether between-subject differences in negative life event and reactivity levels assessed *before* the 12-week follow-up period were associated with subsequent incidence of verified URI. Our hypotheses predict that negative life events and reactivity measures will interact in predicting URI incidence. We also used HLM logistic regression to assess if between-subject differences in SNS, HPA, and immune reactivity levels help explain prospectively who is more likely to experience URI episodes during relatively higher stress weeks. In this case, our hypotheses predicted that weekly perceived stress and reactivity measures would interact in predicting same (or next) week URI episodes. Because of the limitations in the number of verified URI episodes, the HLM procedure could not converge on a solution when analyses were attempted with verified URIs as the outcome variable. Therefore, self-reported URI was used as the outcome variable in all HLM analyses. Furthermore, for the HLM analyses, we used a restricted maximum likelihood estimation method, as this technique can handle missing observations. We specified a variance components model for the parameter covariance matrix. This technique derives independent estimates of sample variance for each intercept and regression coefficient. Because a variance components model assumes that individual differences in slope and intercept terms are unrelated, we mean centered weekly stress values for each participant. Lastly, because repeated measures of an outcome are often correlated with each other (especially those taken closer together in time), we specified a first-order autoregressive error structure to control for autocorrelation.

In all analyses, we controlled for the potentially confounding effects of neuroticism, sex, age, and race. These control variables were entered into the regression first, followed by stress and the reactivity variable of interest, followed by the stress-by-reactivity interaction term. When such interactions were obtained, we dichotomized (median splits) both stress and reactivity and examined the percentage of URI occurrences in each cell of the 2×2 matrix to clarify the nature of the interaction.

RESULTS

Traditional Prospective Analysis of Incidence

Main effects of negative stressful life events and reactivity variables. The results of the logistic regression are presented in Table 1. As apparent from the table, participants displaying dampened CD8⁺ cell number reactivity were more likely than their more reactive counterparts to have a verified URI episode ($b = -0.92, p < .05$). There were no other main effects of reactivity indices or main effects of negative life events in predicting the presence of a verified URI.

Stressful negative life-events-by-reactivity interactions. As apparent from Table 1, there is an interaction between negative life events and cortisol reactivity in predicting the presence of a verified URI. Observed (unadjusted for covariates) percentages of verified URI occurrences for each group in the 2×2 matrix are

TABLE 1. Main Effects of Reactivity Variables and Their Interactions With Negative Life Event Levels Predicting Verified URIs (With Control Variables)

| Predictor Variables | Main Effect and Interaction Statistics Predicting Verified URI | |
|---|--|----------------------------|
| | Main Effect (b) | × Negative Life Events (b) |
| Cortisol reactivity | 0.75** | 5.15* |
| Norepinephrine reactivity | 0.33 | 0.94 |
| Systolic BP reactivity | -0.21 | 0.02 |
| Diastolic BP reactivity | -0.29 | 0.35 |
| Mean arterial pressure reactivity | -0.24 | 0.08 |
| Heart rate reactivity | -0.37 | -0.89 |
| CD8 ⁺ no. reactivity | -0.92* | 0.11 |
| CD4 ⁺ /CD8 ⁺ no. reactivity | 0.31 | 0.83 |
| NK no. reactivity | -0.29 | 3.19 |
| NK %CYT reactivity | -0.35 | 1.99 |

* $p < .05$; ** $p < .10$.

presented in Figure 1. Although both reactivity groups experienced more instances of verified URI when reporting larger vs. smaller numbers of negative life events, this relationship was much more pronounced for the high cortisol reactors. Further, individuals who both reported a large number of negative life events and displayed high cortisol reactivity were more likely to experience a verified URI than the other three groups. None of the other reactivity outcomes were found to interact with negative life events in predicting incidence of verified URI (Table 1).

Week-to-Week Perceived Stress as a Predictor of Week-to-Week URI

Neither current weekly perceived stress levels nor the former week's perceived stress levels were associated with participant reports of an URI during the current week. Results for the main effects of reactivity from the HLM analyses are summarized in Table 2. There was only one significant main effect, with high reactors for changes in CD8⁺ cell number less likely to report an URI than their less reactive counterparts.

Current week's perceived stress interacted with each of the immune reactivities to predict the presence of a current self-reported URI (Table 2). To interpret this result, we performed a median split of each immune reactivity index. We then performed a median split for each participant's 12 weekly perceived stress scores, resulting in a categorization of each person's highest and lowest six scores. We graphed the percentage of weekly observations containing a self-reported URI occurrence for each of the resultant four stress-by-reactivity groups (Figure 2, A to C). In all cases, indi-

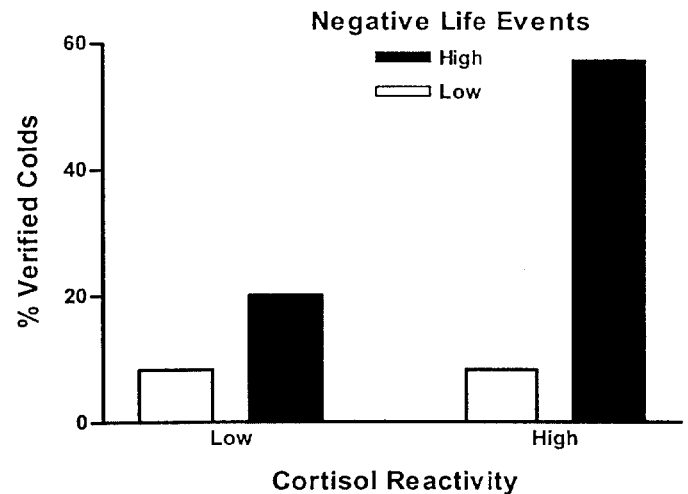


Fig. 1. Percentage of participants who experienced a verified cold episode at any time during the 12-week follow-up period as a function of level of baseline negative life event and cortisol reactivity scores.

viduals who displayed smaller immune reactivity were more likely to experience a self-reported URI when current weekly perceived stress levels were higher vs. lower. Furthermore, in all cases, participants displaying larger increases in immune reactivity exhibited little to no differences in self-reported URI incidence as a function of the current week's perceived stress level. Notably, the graph for the interaction of weekly perceived stress by NK cell number reactivity offers a less clear interpretation of the interaction effect than the others. To elucidate the interaction, we calculated the mean and standard deviation of each participant's 12 weekly PSS score. For each participant, weeks with stress scores greater than or equal to one standard deviation from that participant's mean score were selected to represent high-stress weeks and scores less than or equal to one standard deviation from their mean were selected to represent low-stress weeks. From this new breakdown, it was clearer that low reactors had a slight stress-related increase in URIs (8% in low and 10% in high stress), whereas high reactors had a slight decrease (7% in low vs. 6% in high stress).

There was also a significant interaction between current weekly perceived stress and CD4⁺/CD8⁺ reactivity in predicting current week's self-reported URI. To interpret this result, we performed a median split of CD4⁺/CD8⁺ reactivity and used the low weeks' perceived stress and high weeks' perceived stress groups from above. Those exhibiting high vs. low CD4⁺/CD8⁺ reactivity were more likely to show an increase in self-reported URIs with higher weekly stress levels (Figure 3). Low CD4⁺/CD8⁺ reactors were just as likely

REACTIVITY AND URI

TABLE 2. Main Effects of Reactivity Variables and Their Interactions With Weekly Stress Levels Predicting Weekly Self-Reported URI and URI Symptoms (With Control Variables)

| Predictor Variables | Main Effect and Interaction Statistics Predicting Current URI | | |
|-----------------------------------|---|-------------------|-----------------------|
| | Main Effect (b) | × Current PSS (b) | × Last Week's PSS (b) |
| Cortisol reactivity | 0.02 | -0.01 | 0.03 |
| Norepinephrine reactivity | 0.12 | -0.07+ | -0.02 |
| Systolic BP reactivity | -0.15 | -0.02 | 0.02 |
| Diastolic BP reactivity | -0.17 | -0.04 | 0.04 |
| Mean arterial pressure reactivity | -0.15 | -0.03 | 0.02 |
| Heart rate reactivity | -0.19 | 0.02 | 0.00 |
| CD8+ # reactivity | -0.44* | -0.07* | -0.04 |
| CD4+/CD8+ # reactivity | 0.24 | 0.05* | 0.04 |
| NK # reactivity | -0.10 | -0.06* | -0.02 |
| NK %CYT reactivity | 0.07 | -0.05* | -0.02 |

* $p < 0.05$, ** $p \leq 0.10$.

to report having a URI during lower vs. higher stress weeks, and these rates are comparable with high reactors under low stress. There were no other significant interactions between perceived stress and reactivity in predicting current or lagged self-reported URIs (Table 2).

DISCUSSION

It was predicted that those with larger acute stress-induced increases in cortisol would be more susceptible to URIs under naturalistic stress than their less reactive counterparts. This was supported by the prospective analysis but not the HLM. In the standard prospective analysis, cortisol reactors had a greater incidence of URI than nonreactors among persons with high levels of negative life events. URI incidence was relatively low and unrelated to cortisol reactivity among those with low levels of life events. This result is consistent with the evidence that cortisol suppresses immune function, resulting in less ability to fight off infection, and disrupts the appropriate release of proinflammatory cytokines contributing to excessive symptom expression (31, 32). Because of the prospective nature of the analysis this result cannot be attributed to reverse causation (URIs' triggering increased numbers of stressful life events in cortisol reactors). Moreover, because the URIs were verified, the reported association is likely attributable to the onset of disease as opposed to biases in reporting illness.

We also predicted that greater stress-induced rises in NK cytotoxicity would buffer against stress-associated susceptibility to infectious disease. This hypothesis was not supported by the prospective analysis, but was supported by the HLM analysis. High NK reactors were relatively protected during high stress weeks, but the risk for URIs was low and there was no difference

in risk between high and low reactors on low-stress weeks. This result is consistent with the argument that NK cells are helpful in the face of infectious challenges. There are two limitations to the interpretation of the HLM analyses. First, they were partly cross-sectional (illness and stress) and hence the direction of causation is ambiguous. Second, they were based on unverified reports of illness and hence may reflect biases in reporting of illness rather than difference in underlying pathology. However, there is reason to think that such biases are not a major issue here. Other studies have found very high levels of agreement between self-reported and doctor-verified colds (1). Moreover, neuroticism was controlled for in these analyses, limiting the likelihood that there were psychologically mediated biases in interpretation.

We did not hypothesize whether (or how) immune reactivity as assessed by enumerative markers would moderate the association of naturalistic stressors and URI incidence. Even so, we found that low reactivity of NK number and CD8⁺ cells were both associated with increased risk for URI during weeks when perceived stress was high, but unrelated to URI risk during weeks of low perceived stress. These results are consistent with the argument that both NK and CD8⁺ (T cytotoxic) cells are helpful in the face of infectious challenges.

Finally, we were reluctant to predict whether SNS reactivity would moderate associations between naturalistic stressful events and incidence of URI because SNS activation has been found to be associated with both increases and decreases in immune responses relevant to host resistance. Even so, we were interested in whether we could replicate Boyce's finding in children that blood pressure reactors were at greater risk. None of the SNS markers of reactivity (blood pressure, heart rate, epinephrine, or norepinephrine) interacted

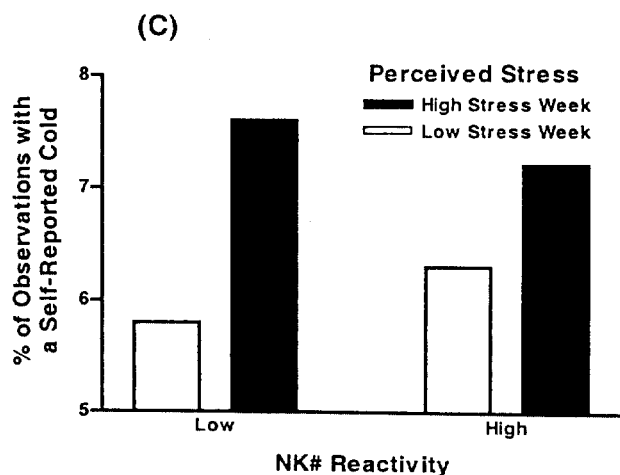
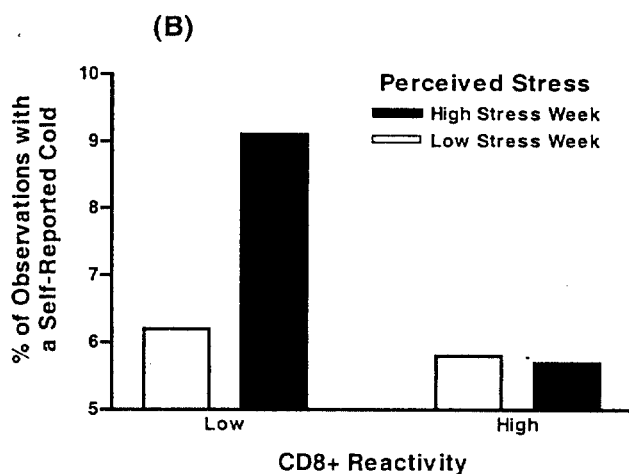
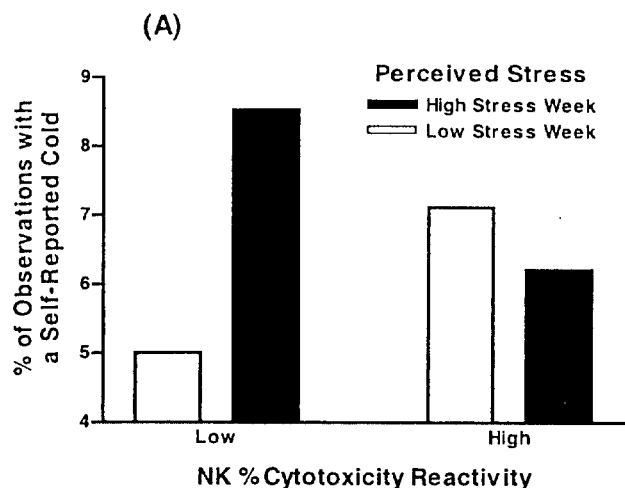


Fig. 2. Percentage of observations (diaries) with a self-reported cold during the current week of the follow-up period as a function of current weekly perceived stress and baseline measures of (A) NK cytotoxicity reactivity; (B) CD8⁺ cell number reactivity; and (C) NK cell number reactivity.

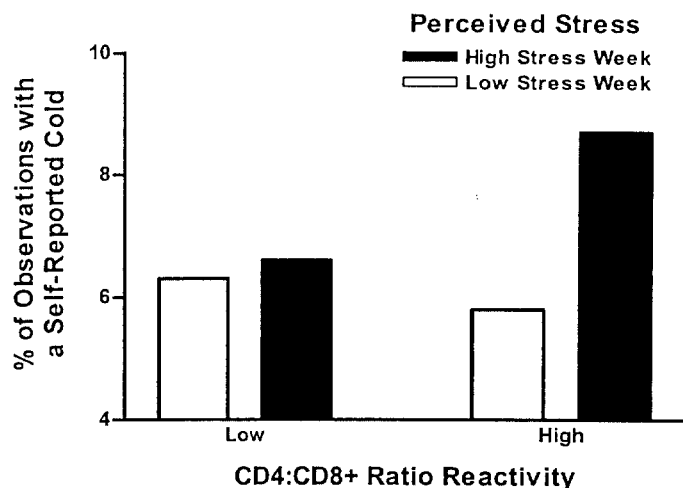


Fig. 3. Percentage of observations (diaries) with a self-reported cold during the current week of the follow-up period as a function of current weekly perceived stress and baseline CD4⁺/CD8⁺ cell number reactivity scores.

with naturalistic stress in either the prospective or HLM analyses.

There are some inconsistencies within our study. Particularly, why did we find different results with the traditional between-subjects prospective analysis of illness incidence and the HLM (within-subject) analysis of the relation between URI episodes and weekly stress levels? In the case of the interactions between stress and immune reactivity, the answer is clear. Although none of these interactions reached significance in the traditional prospective analysis, the patterns of illness rates were all consistent with those found in the within-subject (HLM) analyses. For example, as in the HLM analyses, persons with high life events and low immune reactivity had substantially higher rates of illness (between 38% and 42%) than the remaining subjects in all three cases. It is not surprising that these effects did not reach statistical significance because there is a substantial loss of power in the traditional analyses (relative to the HLM). This is because of the elimination of between-subject error in within-subject analyses and because the traditional prospective analyses focused on verified incidence, which has a much lower base rate of occurrence than the unverified episodes used in the HLM analyses.

In contrast, the interaction between negative life events and cortisol reactivity that we found in the traditional prospective analysis was not found (even in terms of patterns of results) in the HLM analyses. There are several possible reasons. In this case, it is possible that cortisol reactivity is more important in response to the more chronic and important background stressors represented by major stressful life

REACTIVITY AND URI

events than the acute stressful conditions assessed on the weekly level. It is also possible, however, that our results are attributable entirely to individual differences between subjects that would not occur within subjects. For example, the *type of people* who have both high life events and high cortisol reactivity are those at highest risk. This interpretation assumes that both reactivity and having more negative life events are relatively stable characteristics of the person, in contrast to viewing life events more like an environmental factor that is variable across time and relatively independent of the person (33).

Our data are also somewhat inconsistent with data reported by Boyce and his colleagues (5, 6). There are two sources of inconsistency. The first has to do with which biological reactions to stress are reliable across time and hence usable as a marker of a trait like vulnerability factor. Across their three studies, Boyce et al. (5, 6) found that stress-elicited changes in mean arterial pressure (5, study 1), CD19⁺ (5, study 2), CD4⁺/CD8⁺ ratios (6, but not in 5, study 2), and pokeweed mitogen stimulation (6, trend in 5, study 2) interacted with the occurrence of naturalistic stressors in predicting URI. Underlying these results is the assumption that these reactivities are stable across time and task. Although we found that mean arterial blood pressure and CD4⁺/CD8⁺ reactivities were stable over time, B cell reactivity was not. Second, we failed to find an interaction between stress and reactivity of mean arterial pressure (or any blood pressure reactivity measure) in predicting URIs. We did, however, find an interaction of CD4⁺/CD8⁺ reactivity and perceived stress in the HLM analyses. Similar to Boyce et al. (5, 6), we found that during high-stress weeks, high CD4⁺/CD8⁺ reactors were at greater risk. It is noteworthy, however, that in our data, this effect is driven by the CD8⁺ contribution to the ratio. That is, it is another reflection of rises in CD8⁺ reactivity being associated with less risk under stress.

Overall, Boyce and colleagues (5, 6) found that elevated immune reactivity was associated with greater risk under stress. In contrast, we found that elevated immune reactivity was associated with less risk. There are, of course, many differences between the earlier work and our own. Some striking examples include that Boyce et al. studied young children whereas we studied young adults. They assessed the number of URIs experienced within a specific timeframe, whereas we focused on incidence-based analyses that addressed whether stress was associated with at least one occurrence of URI within specific timeframes. (Because our young adults had much fewer total URIs than the children in the Boyce et al. studies (5, 6), we were not able to replicate their type of analyses.) In

their studies, immune reactivities were assessed as responses to a single naturalistic stressor—entering school—with pre- and postmeasures assessed 2 weeks apart. In contrast, we assessed the average response to two laboratory stressors, each over a 5- to 20-minute period. We did not assess mitogen-stimulated proliferation as a measure of reactivity. This measure was, in fact, the one that Boyce and his colleagues found to be a reliable moderator of the stress-illness effect across two studies (5, 6). Because modulation of different immune parameters may have very different implications for host resistance, it is clearly possible that there is less inconsistency here than there seems to be. Moreover, stress-elicited changes in some immune parameters are biphasic, eg, initially rising but after some time falling below basal levels (34). Hence the different direction of associations we found for immune reactivity might be attributable to our assessing immune response at different lags after stress initiation than Boyce and his colleagues.

There was one totally unexpected finding. CD8⁺ reactors had fewer URIs (main effect) in both analyses. In the within-subject (H.L.M.) analysis, this effect may have been just another marker of the magnitude of the interaction; however, in the between-subjects analysis it seems to be a true main effect as there is no indication of an interaction. It might be that CD8⁺ reactivity is a marker of something other than how people respond under stress that is itself important for host resistance, but we are puzzled as to what that might be.

In sum, in prospective analysis, persons showing greater cortisol reactivity to acute stressors had increased risk for verified URI when naturalistic levels of stress were also high. In contrast, those with smaller cortisol reactivity showed little to no stress-associated increase in URI incidence. We attributed this finding to cortisol's compromising immune competence. Similarly, in the cross-sectional (H.L.M.) analyses, smaller immune responses under acute stress were associated with increased risk for URI during stressful periods. This is also supportive of stress-induced immunity (at least NK cytotoxicity and increase in circulating CD8⁺ cells) as protective in the face of stressful events. These data are supportive of the proposal that laboratory-assessed endocrine and immune reactivities indicate vulnerability to stress-associated infectious illness in the natural environment.

This research was supported by grants from the National Institute of Mental Health (MH50429, MH50430), by supplements from the National Institutes of Health Office of Behavioral and Social Sciences, by the John D. and Catherine T. MacArthur Foundation Network on Socioeconomic Status and

Health, and by a grant from the National Institutes of Health to the University of Pittsburgh Medical Center General Clinical Research Center (NCRR/GCRC 5M01 RR00056). Dr. Cohen's participation is supported by a Senior Scientist Award from the National Institute of Mental Health (MH00721). We are indebted to Erik Jacobson, Laurie Nelson, Anna Marsland, Janet Schlarb, Anita Barkin, Howard Seltman, Clemens Kirschbaum, the staff of the General Clinical Research Center for their contributions to this research effort, and to the Pittsburgh Mind-Body Center for facilitating this collaboration (HL65111 and HL65112).

REFERENCES

- Cohen S, Tyrrel DAJ, Smith AP. Psychological stress and susceptibility to the common cold. *N Engl J Med* 1991;325:606-12.
- Turner Cobb JM, Steptoe A. Psychosocial stress and susceptibility to upper respiratory tract illness in an adult population sample. *Psychosom Med* 1995; 58:404-12.
- Clover RD, Abell T, Becker LA, Crawford S, Ramsey CN. Family functioning and stress as predictors of influenza B infection. *J Fam Pract* 1989;28:535-39.
- Stone AA, Bovbjerg DH, Neale JM, Napoli A, Valdimarsdottir H, Cox D, Hayden FG, Gwaltney JM. Development of common cold symptoms following experimental rhinovirus infection is related to stressful life events. *Behav Med* 1992;18:115-20.
- Boyce WT, Chesney M, Alkon A, Tschann JM, Adams S, Chesterman B, Cohen F, Kaiser P, Folkman S, Wara D. Psychobiologic reactivity to stress and childhood respiratory illnesses: results of two prospective studies. *Psychosom Med* 1995;57:411-22.
- Boyce WT, Chesterman EA, Martin N, Folkman S, Cohen F, Wara D. Immunologic changes occurring at kindergarten entry predict respiratory illnesses following the Loma Prieta earthquake. *J Dev Behav Pediatr* 1993;14:296-303.
- Cohen S, Manuck SB. Stress, reactivity and disease. *Psychosom Med* 1995;57:423-26.
- Manuck SB. Cardiovascular reactivity in cardiovascular disease: "once more unto the breach." *Int J Behav Med* 1994;1:4-31.
- Cohen S, Hamrick N, Rodriguez MS, Feldman PJ, Rabin BS, Manuck SB. The stability of and intercorrelations among cardiovascular, immune, endocrine, and psychological reactivity. *Ann Behav Med* 2000;22:1-10.
- Kamarck TW, Jennings JR, Manuck SB. Psychometric applications in the assessment of cardiovascular reactivity. *Homeostasis* 1993;34:229-43.
- Mills PJ, Berry CC, Dimsdale JE, Nelesen RA, Ziegler MG. Temporal stability of task-induced cardiovascular, adrenergic, and psychological responses: the effects of race and hypertension. *Psychophysiology* 1993;30:197-204.
- Kirschbaum C, Prussner JC, Stone AA, Federenko I, Gaab J, Lintz D, Schommer N, Hellhammer DH. Persistent high cortisol responses to repeated psychological stress in a subpopulation of healthy men. *Psychosom Med* 1995;57:468-74.
- Kuhn CM. Adrenocortical and gonadal steroids in behavioral cardiovascular medicine. In: Schneiderman N, Weiss SM, Kaufmann PG, editors. *Handbook of research methods in cardiovascular behavioral medicine*. New York: Plenum Press; 1989. p. 185-204.
- Dhabhar FS, McEwen BS. Bidirectional effects of stress and glucocorticoids hormones on immune function: Possible explanation for paradoxical observations. In: Ader R, Felten DL, Cohen N, editors. *Psychoneuroimmunology*, 3rd edition, Volume 1. New York: Academic Press; 2001. p. 301-38.
- Sheridan JF. Norman Cousins Memorial Lecture 1997: Stress-induced modulation of anti-viral immunity. *Brain Behav Immun* 1998;12:1-6.
- Herbert TB, Cohen S, Marsland AL, Bachen EA, Muldoon MF, Rabin BS, Manuck SB. Cardiovascular reactivity and the course of the immune response to an acute psychological stressor. *Psychosom Med* 1994;56:337-44.
- Manuck SB, Cohen S, Rabin BS, Muldoon MF, Bachen E. Individual differences in cellular immune response to stress. *Psychol Sci* 1991;2:111-5.
- Bachen EA, Manuck SB, Cohen S, Muldoon MF, Raible R, Herbert TB, Rabin BS. Adrenergic blockade ameliorates cellular immune responses to mental stress in humans. *Psychosom Med* 1995;57:366-72.
- Marsland A, Manuck SB, Fazzari TV, Stewart C. Stability of individual differences in cellular immune responses to acute psychological stress. *Psychosom Med* 1995;57:295-8.
- Zakowski SG, McAllister CG, Deal M, Baum A. Stress, reactivity, and immune function in healthy men. *Health Psychol* 1992;11:223-32.
- Mills PJ, Haeri SL, Dimsdale JE. Temporal stability of acute stressor-induced changes in cellular immunity. *Int J Psychophysiol* 1995;19:287-90.
- Munck A, Guyre PM. Glucocorticoids and immune function. In: Ader R, Felten DL, Cohen N, editors. *Psychoneuroimmunology*, 2nd edition. New York: Academic Press; 1991. p. 447-474.
- Benschop RJ, Nieuwenhuis EES, Tromp EAM, Godaert GLR, Bailieux RE, Van Doornen LJP. Effect of β -adrenergic blockade on immunologic and cardiovascular changes induced by mental stress. *Circulation* 1994;89:762-9.
- Feldman P, Cohen S, Doyle WJ, Skoner DP, Gwaltney JM. The impact of personality on the reporting of unfounded symptoms and illness. *J Pers Soc Psychol* 1999;77:370-8.
- Pennebaker JW. *The psychology of physical symptoms*. New York: Springer-Verlag 1982.
- Fletcher MA, Baron GC, Ashman MR, Fischl MA, Klimas NG. Use of whole blood methods in assessment of immune parameters in immunodeficiency states. *Diagn Clin Immunol* 1987;5:69-81.
- Kirschbaum C, Hellhammer DH. Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology* 1989;22:150-69.
- Henderson S, Byrne DG, Duncan-Jones P. *Neurosis and the social environment*. San Diego: Academic Press; 1981.
- Eysenck HJ, Eysenck SBG. *Manual of the Eysenck Personality Inventory*. London: University of London Press; 1964.
- Cohen S, Williamson G. Perceived stress in a probability sample of the United States. In: Spacapan S, Oskamp S, editors. *The social psychology of health: Claremont symposium on applied social psychology*. Newbury Park: Sage; 1988:31-67.
- Cohen S, Doyle WJ, Skoner DP. Psychological stress, cytokine production, and severity of upper respiratory illness. *Psychosom Med* 1999;61:175-80.
- Miller GE, Cohen S, Ritchey AK. Chronic psychological stress and the regulation of pro-inflammatory cytokines: A glucocorticoids resistance model. Unpublished manuscript; 2001.
- McFarlane AH, Norman GR, Streiner DL, Roy RG. The process of social stress: stable, reciprocal, and mediating relationships. *J Health Soc Beh* 1983;24:160-73.
- Delahanty DL, Wang T, Maravich C, Forlenza M, Baum A. Time-of-day effects on response of natural killer cells to acute stress in men and women. *Health Psychol* 2000;19:39-45.