Psychological Stress, Cytokine Production, and Severity of Upper Respiratory Illness

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Objective: The purpose of this study is to assess the role of psychological stress in the expression of illness among infected subjects and to test the plausibility of local proinflammatory cytokine production as a pathway linking stress to illness. **Methods:** After completing a measure of psychological stress, 55 subjects were experimentally infected with an influenza A virus. Subjects were monitored in quarantine daily for upper respiratory symptoms, mucus production, and nasal lavage levels of interleukin (IL)-6. **Results:** Higher psychological stress assessed before the viral challenge was associated with greater symptom scores, greater mucus weights, and higher IL-6 lavage concentrations in response to infection. The IL-6 response was temporally related to the two markers of illness severity, and mediation analyses indicated that these data were consistent with IL-6 acting as a major pathway through which stress was associated with increased symptoms of illness. However, this pattern of data is also consistent with increases in IL-6 occurring in response to tissue damage associated with illness symptoms. **Conclusions:** Psychological stress predicts a greater expression of illness and an increased production of IL-6 in response to an upper respiratory infection. **Key words:** cytokines, IL-6, psychological stress, infection, upper respiratory illness, influenza.

IL-6 = interleukin-6; HI = hemagglutination-inhibition; TNF = tumor necrosis factor; PSS-10 = 10-item Perceived Stress Scale.

INTRODUCTION

Exposure to viral agents that cause upper respiratory disease provokes illness in some individuals but not others. Moreover, the severity of symptomatology among those who develop illness varies substantially. Evidence from prospective epidemiological studies (eg, Refs. 1–5) and from experimental viral-challenge studies (6–10) show that persons reporting more psychological stress have both a higher incidence and a greater severity of illness. However, past attempts to identify the behavioral and biological pathways linking psychological stress with upper respiratory viral illness have been unsuccessful (5–7).

Recent studies reported that psychological stressors acutely activate the production of the proinflammatory cytokine IL-6 (11). In response to infection, IL-6 release is thought to be mediated by glucocorticoids, thus providing a hypothetical pathway by which stressors (via their induction of glucocorticoid production) could control cytokine release (12). In addition, IL-6 triggers additional release of glucocorticoids, possibly exacerbating the stress response by positive feedback modulation. At least one source of IL-6 is epithelial cells as evidenced by their production of IL-6 in vitro and in vivo when exposed to rhinovirus (13). Because a local increase in the concentration of this proinflammatory cytokine precedes the development of acute signs and symptoms of illness, it has been implicated as a mediator in the pathway for symptom expression. In fact, IL-6 concentrations in nasal secretions were associated with upper respiratory symptoms among persons infected with influenza A virus [A/Texas and A/Kawasaki (14, 15)] and rhinovirus [strain Hanks' and type 39 (13)].

In the present study, we address the hypothesis that IL-6 production in response to influenza A virus infection represents a viable pathway through which psychological stress influences the severity of illness. To achieve that goal, we measured levels of psychological stress in a group of adult subjects before infecting them with an influenza A virus, and measured self-reported respiratory symptoms, mucus weights, and local IL-6 concentrations on the day before and for 7 days after virus exposure. We tested the hypotheses that the level of psychological stress predicts the severity of illness and also the magnitude of the cytokine response, and then examined the data for evidence of IL-6 mediation of the association between stress and illness.

METHODS

Subjects

Subjects were adult volunteers who were recruited by advertisement. Persons were excluded from participation in the study if they: a) had a history of seizures, nasal or otological surgery, pulmonary, cardiovascular, renal, or other serious diseases; b) had a symptomatic upper respiratory infection within the previous 30 days or otitis media within the last 6 months; c) were pregnant on presentation; or d) had nasal or otological signs and symptoms, abnormal clinical profiles, seropositivity for human immunodeficiency virus or a hem-

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Received for publication August 12, 1998; revision received December 3, 1998.

0033-3174/99/6102-0175

agglutination-inhibition (HI) antibody titer of >1:10 to influenza A/Kawasaki/86 H1N1 virus. Seventy-five adults were enrolled in the study, of whom 55 had complete data for all study variables and met the criteria for infection by the challenge virus. These 55 subjects represent the sample for this analysis. All subjects gave written informed consent for HIV testing and study participation, and the protocol was approved by the Human Rights Committee at the Children's Hospital of Pittsburgh.

Design

The study was run in two approximately equal cohorts, one in September and the other in December. Subjects were quarantined for a total of 8 days in a local hotel. During the first 24 hours of quarantine (before viral exposure), they had a complete physical examination, a nasal wash culture for Kawasaki A virus and each woman had a pregnancy test. Subjects were excluded if there was any indication of recent or current upper respiratory infection, illness, or pregnancy. On that day, they also completed the Perceived Stress Scale and recorded baseline respiratory symptoms. Expelled nasal secretions were collected and weighed and a nasal lavage was performed for baseline viral culture and assay of local IL-1, IL-6, and tumor necrosis factor (TNF) concentrations.

At the end of the first 24 hours of quarantine, subjects were given nasal drops containing an infectious dose (0.25 ml inoculum containing approximately 10^7 $TCID_{50}$) of a safety-tested clinical isolate of influenza A/Kawasaki/86 H1N1 (wild type, lot E-262; NIH). Beginning 48 hours after inoculation and continuing for the remainder of the quarantine period, subjects were treated twice a day according to a randomization code with either oral rimantadine HCL (Flumadine, 100-mg tablets; Forest Pharmaceuticals, St Louis, MO), or an identically appearing lactose placebo. This was done in accordance with the protocol of the parent NIH funded study from which this population was drawn (16). On all days, nasal lavages for virus culture and assay of cytokine levels were collected, subjects rated the severity of their respiratory symptoms, and nasal mucus production was measured. Approximately 28 days after challenge, a blood sample was collected for serological testing of HI antibody titer to the challenge virus. All investigators were blinded to treatment and scores on the Perceived Stress Scale.

Perceived Stress Scale

The 10-item Perceived Stress Scale (PSS-10) was used to assess the degree to which situations in life are perceived as stressful (reliability Cronbach's $\alpha = .87$ in this sample). Items in the PSS-10 were designed to tap how unpredictable, uncontrollable, and overloading respondents find their lives (17).

Criteria for Infection

Because we were interested in the role of stress and proinflammatory cytokines in triggering symptomatology, only the 55 subjects infected with the virus were included in our analyses. Subjects were classified as infected if the challenge virus was isolated in nasal washings on any of the 7 postinoculation study days or if there was at least a 4-fold increase in virus-specific HI antibody titer. For culture, samples of lavage fluid were inoculated onto Madin-Darby canine kidney cell monolayers in triplicate and the virus was identified by hemadsorption. The first isolate was confirmed to be influenza by immunofluorescent testing (18). Antibody titers were assessed by hemagglutination-inhibition testing using standard techniques (19).

Signs and Symptoms

At the end of each day of quarantine, subjects rated the severity of eight respiratory symptoms (sneezing, nasal discharge, nasal congestion, sore throat, cough, malaise, headache, chilliness) during the previous 24 hours. Ratings ranged from 0 (none) to 3 (severe) for each symptom. The symptom scores were summed within day. Mucus production was assessed by collecting used tissues in sealed plastic bags. The bags were weighed and the weight of the tissues and bags subtracted. Mucus weights were also summed within day. Because the distribution of mucus weights was skewed, we used the $\log_{10}$ in all analyses.

Proinflammatory Cytokines

Cytokines were assayed using ELISAs obtained from Endogen (Woburn, MA). All had inter- and intraassay variabilities of <10%. The lower limit of detectability was 5 pg/ml for TNFα, 1 pg/ml for IL-1β, and 1 pg/ml for IL-6. We could not reliably detect either IL-1β or TNFα in nasal secretions at baseline or on any day after virus inoculation. Hence, the data analysis was confined to IL-6 levels. Because the distribution of IL-6 was skewed, we used the $\log_{10}$ in all analyses.

RESULTS

Analyses

Control Variables. We assessed several variables thought to be associated with both psychological stress and disease expression, and hence might provide alternative explanations for any observed relationships between stress and disease. These included age, gender, race, body mass (weight/height^2), and cohort (September or December). Preliminary analyses indicated that only body mass was associated with *both* psychological stress and any of the outcome variables related to disease expression (using liberal α of $p < .20$). Therefore, body mass was included in all analyses as a covariate.

Statistical Model. The primary dependent variables were the total scores for each disease outcome summed across the 7 days after inoculation. We also report separate analyses for each outcome on each day of the trial. In all analyses the postinoculation symptoms, mucus weights, and cytokine levels are adjusted for baseline. In bivariate correlations, this adjustment is accomplished by using residualized change scores—regressing the postchallenge total of each outcome on its corresponding baseline level and calculating the residual score. The residual score is the difference between what one would predict from baseline and the observed score. Identical adjustment is accomplished in the multiple regression analyses by including baseline measures as covariates.

The major hypotheses were tested using multiple regressions. Independent variables entered into the regression equations included psychological stress

(treated as a continuous variable), receipt of active medication (yes or no), followed by the stress-by-medication interaction. Symptoms, mucus weights, and IL-6 levels were analyzed as separate dependent variables. The baseline (day 0) levels of the outcome variables were controlled for by forcing the appropriate (same as outcome) baseline measure into the regression before the independent variables. Body mass was similarly covaried in all analyses. The regression analysis allowed us to maintain the continuous character of psychological stress as well as to directly and simply test hypotheses about potential pathways. To provide an estimate of the effect size of each association, we present the appropriate partial correlation with each F statistic. The partial correlation represents the association between stress and a specific outcome after controlling for baseline, receipt of active medication or not, and body mass.

Figures 1 through 3 summarize the associations between psychological stress and each of the three outcome variables. To simplify the presentation, psychological stress was dichotomized in these figures (median = 14). However, all of the analyses we report treated stress as a continuous variable.

Psychological Stress and Symptoms

The relation between psychological stress and self-reported symptoms of upper respiratory illness is depicted in Figure 1. Overall, symptoms increased sharply to peak 2 days after inoculation and then decreased to the prechallenge, baseline level by day 7. Total symptom scores after inoculation (controlling for baseline symptoms) increased with increasing levels of psychological stress ($F(1,50) = 6.0$, $p < .02$; $r = .33$). Symptom scores were not associated with either medication or the psychological stress-by-medication interaction. Analyses of individual days indicate relations between greater stress and greater symptoms on days 2 ($F(1,50) = 5.8$, $r = 0.32$, $p < .02$) and 3 ($F(1,50) = 4.9$, $r = .30$, $p < .04$). Marginal associations were found for days 4 ($F(1,50) = 3.0$, $r = .24$, $p < .10$) and 5 ($F(1,50) = 3.4$, $r = .25$, $p < .07$). There were no associations on other days.

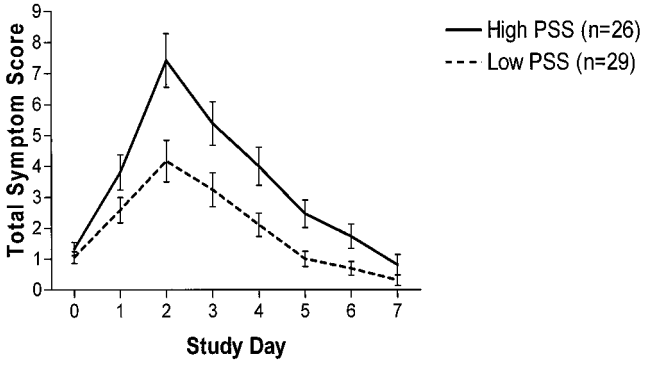

Fig. 1. The association between psychological stress (low = below median and high = above median) and symptoms of upper respiratory illness among subjects infected with an influenza A virus. Viral inoculation occurred at the end of day 0. SEs are indicated.

Psychological Stress and Mucus Weights

The relation between psychological stress and mucus weights is depicted in Figure 2. As with symptoms, mucus weights increased sharply to peak 2 to 3 days after inoculation and then decreased to their baseline level by day 7. Mucus weights after inoculation (controlling for baseline) increased with increasing levels of psychological stress ($F(1,50) = 6.3$, $p < .02$; $r = .33$). Mucus weights were not associated with either medication or the psychological stress-by-medication interaction. Analyses of individual days indicate relations between stress and mucus weights on days 2 ($F(1,50) = 8.7$, $r = .38$, $p < .01$), 3 ($F(1,50) = 7.1$, $r = .35$, $p < .01$), and 4 ($F(1,50) = 9.7$, $r = .40$, $p < .01$). There were no associations on other days.

Psychological Stress and IL-6

The relation between psychological stress and local IL-6 concentration is depicted in Figure 3. A sharp increase in IL-6 levels occurred over the first 2 days after inoculation, but unlike the pattern for symptoms and mucus weights, IL-6 levels remained slightly elevated not returning to baseline levels during the period of follow-up. IL-6 levels after inoculation (controlling for baseline) increased with increasing psychological stress scores ($F(1,50) = 5.8$, $p < .02$, $r = .32$). IL-6 levels were not associated with either medication or the psychological stress-by-medication interaction. Analyses of individual days indicate that IL-6 increases with

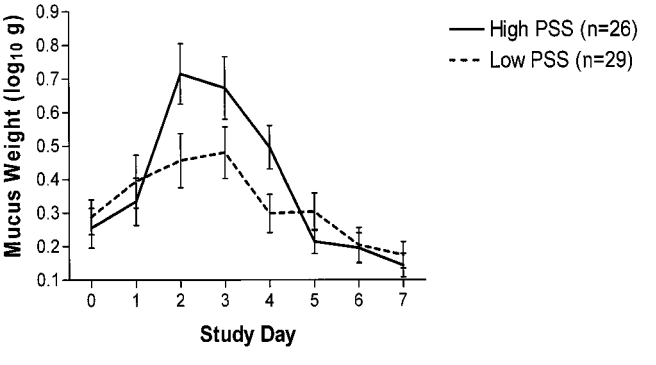

Fig. 2. The association between psychological stress (low = below median and high = above median) and mucus weights among subjects infected with an influenza A virus. Viral inoculation occurred at the end of day 0. SEs are indicated.

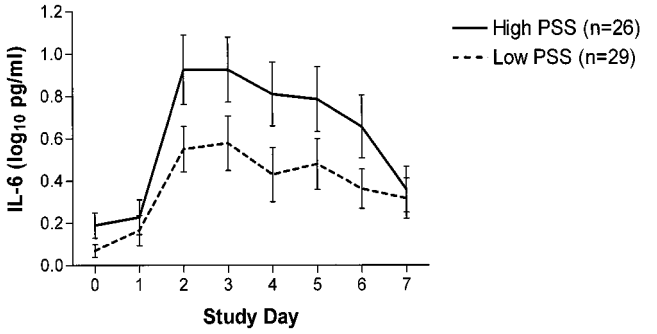

Fig. 3. The association between psychological stress (low = below median and high = above median) and IL-6 in nasal secretions among subjects infected with an influenza A virus. Viral inoculation occurred at the end of day 0. SEs are indicated.

increased stress on days 2 ($F(1,50) = 4.9$, $r = .30$, $p < .04$), and 4 ($F(1,50) = 6.5$, $r = .34$, $p < .02$). Marginal associations occurred on days 3 and 5 (in both cases $F(1,50) = 3.1$, $r = .24$, $p < .09$). There were no associations on other days.

There was a 4.7-fold difference in pg/ml between those less than (7.4 pg/ml) and more than (35.0 pg/ml) the median on perceived stress on day 2 (largest difference). Summing across the 7 postinoculation days, there was a 2.3-fold increase (61.8 pg/ml for those below the median and 140.1 pg/ml for those above). The magnitude of these differences is consistent with the IL-6 changes in drug treatment trials with documented physiological effects (20, 21).

IL-6 as a Pathway Linking Psychological Stress to Disease

Although the correlational nature of these data does not allow for a direct test of whether the documented association between stress and disease expression is mediated by IL-6, we can examine the extent to which such mediation is consistent with the data. Conditionally, our hypothesis is consistent if: a) stress is associated with the proposed mediator; b) the proposed mediator is associated with the outcome; and c) the relation between stress and outcome is substantially reduced by partialing the effect of the mediator out of the correlation between stress and the outcome (22). Condition 1 is satisfied by our observation that persons with higher levels of psychological stress produce more IL-6 ($r = .32$, $p < .02$), whereas condition 2 is satisfied by the significant correlations calculated for IL-6 and symptoms ($r = .38$, $p < .005$) and for IL-6 and mucus weights ($r = .46$, $p < .001$). Finally, adding total postinoculation IL-6 levels as a covariate in the analyses reduced the percent variance in symptoms explained by perceived stress from 8.8% in the original analysis to 3.7%, and reduced the percent explained variance in mucus weight from 8.2% to 2.7%. Moreover, perceived stress no longer predicts symptoms ($F(1,49) = 2.8$, $p > .10$) or mucus weights ($F(1,49) = 2.5$, $p > .10$) after IL-6 was entered as a covariate. Thus, after partialing out the effect of IL-6, the effect size of psychological stress on symptoms was reduced by 58% (1–3.7/8.8) and that of psychological stress on mucus weight by 67% (1–2.7/8.2). These results satisfy condition 3 and show that our data are consistent with the hypothesis that IL-6 is a primary mediator of the relationship between stress and illness severity.

Similar analyses were conducted separately for days 2, 3, and 4. As reported earlier, stress was associated with symptoms, mucus weights, and IL-6 on these days. When IL-6 was partialed out of the relation between stress and symptoms, the effect size was reduced 92% (1–0.7%/8.7%) on day 2, 65% (1–2.8%/8.0%) on day 3, and 94% (1–0.3%/5%) on day 4. When IL-6 was partialed out of the relation between stress and mucus weights, the effect size was reduced 72% (1–3.0%/10.8%) on day 2, 65% (1–3.3%/9.4%) on day 3, and 44% (1–8.1%/14.4%) on day 4.

DISCUSSION

The results we report are consistent with those of earlier studies documenting that persons with higher levels of psychological stress express more symptoms and signs of upper respiratory infectious illness (see review in Ref. 23). In this study, all subjects had verified infection with the same strain of influenza A virus, and the association between psychological stress and illness was documented for both subjective reports of symptoms and objective measures of mucus production. The concurrent use of these two markers of illness shows that psychological stress is associated with aspects of the actual underlying disease and not simply the subjects' perceptual representations of illness.

We also found that during the early period of influenza A virus infection, the local concentration of the proinflammatory cytokine IL-6 varied in phase with the symptoms and mucus production provoked by the infection. This observation is consistent with earlier work showing a temporal relationship between IL-6 levels and symptoms for upper respiratory infection with rhinovirus (13) and with influenza A virus (14), and suggests that IL-6 production may represent a common pathway through which upper respiratory virus infection is translated into signs and symptoms. Furthermore, persons reporting greater psychological stress before inoculation responded to infection with a greater concentration of this cytokine in their nasal

secretions. This association between stress and cytokine production is consistent with earlier work in both animals and humans (24). It is noteworthy, however, that in our data the association between baseline levels of IL-6 and psychological stress did not reach significance ($r = .24$, $p < .09$). It was the IL-6 response to the viral infection (controlling for baseline) that was significantly higher for those reporting greater psychological stress. That the relation between stress and IL-6 was marginal without the stimulus of the infectious challenge is probably attributable to a restricted range of variance in IL-6 levels under basal conditions. It could also be attributable to greater measurement error (and hence less statistical power) when basal levels are based on only one sample. A similar point could be made about several marginal effects we found when looking at individual postinoculation days of the trial.

We hypothesized that increased nasal secretory production of IL-6 might mediate the relationship between increased psychological stress and increased signs and symptoms of illness. Our analyses suggest that most of the effect of psychological stress on symptoms and of psychological stress on mucus weights could be accounted for by changes in IL-6. However, it is possible that IL-6 itself is not the causal link but rather just a marker (covariate) of other unassayed proinflammatory chemicals elevated during the course of experimental infection. For example, Hayden et al. (14) reported that interferon α and IL-6 levels (but not TNF, IL-8, IL-1, or IL-2) were both increased early in the course of the infection, and that both correlated with viral titers, temperature, mucus production and symptom scores. There is also an issue of the correlational nature of the mediational analysis. Although consistent with the hypothesis that the association of stress and illness are mediated by IL-6, the data do not permit causal inference. For example, this pattern of data is also consistent with increases in IL-6 occurring in response to tissue damage associated with illness symptoms. Even with these reservations, this is the first study to provide evidence consistent with the hypothetical model that psychological stress influences upper respiratory infectious illness through a biological pathway.

Finally, there is the question of why symptoms and mucus weights decrease at faster rates than IL-6 concentrations. As mentioned earlier, proinflammatory cytokines are both a product of and producer of the inflammatory process. The slower return of IL-6 levels in the high PSS group (Figure 3) could be a residual marker of the tissue damage occurring in the individuals with greater signs and symptoms of illness.

This work was supported by Grant MH50429 and a Senior Scientist Award, Grant MH00721, from the National Institute of Mental Health and by Grant DC02833 from the National Institutes of Health. Supplemental support was provided by the John D. and Catherine T. MacArthur Foundation Network on Socioeconomic Status and Health. Challenge virus was supplied by Brian R. Murphy under a Material Transfer agreement with the National Institute of Allergy and Infectious Diseases.

We are indebted to Janet Schlarb, James Seroky, Cuneyt Alper, Gregory Allen, Stephanie Moody, Patricia Fall, and Susan Strelinski for their assistance in conducting this study. We acknowledge Frederick Hayden and Carolyn Crump for performing and interpreting the virological aspects of the study, Asha Patel for performing the cytokine assays, and Bruce S. Rabin for his comments on an earlier draft.

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