

Emotional style, nasal cytokines, and illness expression after experimental rhinovirus exposure

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Abstract

Psychosocial factors moderate the expression of illness during upper respiratory virus infections but past attempts to define mediational pathways were not successful. Here, we used a model of experimental rhinovirus infection in humans to evaluate three proinflammatory cytokines for their potential role in mediating the previously documented association between positive emotional style and illness. After assessing emotional style in 327 healthy adults, each was exposed to one of two strains of rhinovirus and followed for 5 days in quarantine. Symptoms/signs, nasal lavage IL-1 β , IL-6, and IL-8 protein, and viral shedding were assessed at baseline and on each of the 5 days after exposure. Virus-specific antibody was assessed at baseline and 28 days after challenge. An analysis of the data for 234 subjects with documented infection showed that nasal IL-1 β , IL-6, and IL-8 protein levels were all associated with greater illness expression but IL-6 was by far the best predictor of nasal signs and symptoms. Lower positive emotional style was associated with greater objective and subjective markers of illness and these associations were decreased substantially by controlling for IL-6 but not for IL-1 β or IL-8. These results are consistent with the hypothesis that IL-6 acts as a biological mediator in linking positive emotional style to illness expression during rhinovirus infection.

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1. Introduction

A traditional method for studying the pathogenesis of upper respiratory diseases and their complications is the assay of local secretions and/or other recovered fluids for chemicals that are expected to participate in the immune/inflammatory process (Doyle et al., 2005). High levels of proinflammatory and anti-inflammatory cytokines including interleukin (IL)-1 β , IL-6, IL-8, IL-10, interferon (INF) α , INF γ , and tumor necrosis factor (TNF) α have been recovered in nasal secretions and nasal lavage fluids at the time of acute viral upper respi-

ratory illnesses (vURIs) caused by respiratory syncytial virus, parainfluenza virus, rhinovirus (RV), influenza virus, and infections of unspecified etiology (Chen et al., 2002; Garofalo et al., 2004; Gern et al., 2002; Hornsleth et al., 2001; Kaiser et al., 2001; Noah et al., 1995; Oh et al., 2002; Sheeran et al., 1999). Assays of nasal cytokine production in adults experimentally infected with known viruses indicated similarly patterned cytokine responses that were consistent across viruses (de Kluijver et al., 2003; Doyle et al., 2005; Fritz et al., 1999; Hayden et al., 1998; Linden et al., 1995; Noah and Becker, 2000; Yoon et al., 1999; Yuta et al., 1998; Zhu et al., 1996, 1997). For example, Hayden and colleagues reported increases in nasal IL-6, IL-8, TNF α , and INF α levels in persons expressing symptoms after influenza

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challenge (Hayden et al., 1998) and Gentile and colleagues reported increased levels of IL-1 β and IL-6 in only those subjects who expressed symptoms after RV challenge (Gentile et al., 2003).

A continuing focus of our research is the psychosocial modifiers of illness expression during natural and experimental vURIs. For example, we previously reported that chronic stress, less diverse social networks, and low levels of positive emotional style (PES) all predicted an increased probability of developing a common cold in persons experimentally infected with an upper respiratory virus (e.g., Cohen et al., 1991, 1997, 1998, 1999, 2003). However, while most pathways (e.g., cortisol, epinephrine and norepinephrine, cigarettes/day, alcoholic drinks/day, zinc, and vitamin C intake) evaluated as potentially linking these psychosocial factors to illness severity were not supported, the results of one study suggested a mediating role for local IL-6 production (Cohen et al., 1999). There, the symptom/sign response and nasal IL-6 level for 55 adult subjects experimentally infected with influenza A virus were monitored and compared between groups defined by pre-exposure perceived stress. The results documented that greater stress predicted greater IL-6 production, symptoms and nasal secretions, and mediational analysis supported IL-6 production as a potential pathway linking perceived stress to illness (Cohen et al., 1999).

A role for IL-6 in mediating the association between psychosocial factors and illness is supported by the results of past studies. Basal serum levels of IL-6 are higher in subjects reporting negative psychological traits and behaviors, and lower in those reporting positive ones. For example, serum levels of IL-6 were higher in those who reported high levels of hostility (Suarez, 2003) and anger (Lutgendorf et al., 1999), and lower in cancer patients seeking social support (Lutgendorf et al., 2000) and in individuals who reported frequent church attendance (Koenig et al., 1997). Moreover, in an animal model, the release of IL-1, TNF α , and IL-6 during infection was reported to be modulated by glucocorticoids, thus providing a hypothetical pathway by which psychosocial factors (via their influence on glucocorticoid production) could control cytokine production (Dobbs et al., 1996; Konstantinos and Sheridan, 2001).

PES is a measure of dispositional positive affect and low levels of PES have been implicated as contributing to greater mortality among community-dwelling elderly, higher morbidity for several diseases, and greater reports of unfounded pains and symptoms (review in Pressman and Cohen, *in press*). We previously reported a protective effect of high PES on cold incidence in a large cohort of adult subjects experimentally infected with RV39 or RV23 (Cohen et al., 2003) and more recently assayed archived, serial nasal lavage samples collected from these subjects for IL-1 β , IL-6, and IL-8 protein levels. This panel of cytokines was chosen for assay because its

members are locally produced during the ill period of a vURI (Gentile et al., 2003; Hayden et al., 1998); were suggested to directly mediate symptom/sign expression (Cohen et al., 1999; Doyle et al., 2005), and their serum levels have been related to a wide range of psychosocial factors such as depression (Kubera et al., 2000), hostility (Suarez, 2003), and church attendance (Koenig et al., 1997).

In this report, we evaluated the hypothesis, developed from our study of IL-6 production in influenza infected subjects, that PES modulates illness during RV infection via an intermediate effect on local IL-6 production. By using three distinct symptom clusters and two objective signs of illness as outcomes, we also tested if each of the assayed cytokines—IL-1 β , IL-6, and IL-8—mediates a specific domain of symptoms/signs and if the effects of PES on illness are specific to a symptom/sign domain. Because the majority of outcomes are self-reported symptoms, we included a group of control symptoms (not characteristic of a vURI) that are expected to be subject to reporting bias but not influenced by experimental conditions.

2. Materials and methods

Adult subjects (>18 years) were recruited by advertisements from the metropolitan Pittsburgh, PA area. On presentation for screening, the design and obligations of study participation were explained and informed consent was obtained from those interested in participating. All consented volunteers underwent medical screenings by history and physical, supplied demographic information and had blood taken for assay of pre-exposure RV-specific neutralizing antibodies. Subjects were excluded on screening if: (1) they had a history of psychiatric illness, major nasal or otologic surgery, asthma or cardiovascular disorders, (2) they were on a regular medication regimen for a chronic illness, (3) their results for urinalysis, complete blood counts, or blood enzymes were abnormal, (4) they were pregnant or currently lactating, or (5) they were seropositive for human immunodeficiency virus. During the 6-week interval between screening and virus exposure, each subject's baseline positive and negative emotional styles were assessed using methods described below.

Then, participating subjects presented to a local hotel for a 6-day period of quarantine. During the first 24 h (day 0, pre-exposure), female subjects had a rapid pregnancy test and all subjects: (1) had an ENT/physical examination, a nasal mucociliary clearance test, and a nasal lavage for virus and cytokine assay; (2) collected their nasal secretions in pre-weighed tissues, and (3) completed a symptom diary. Volunteers were excluded from continuation if they were pregnant or had signs/symptoms of a cold-like illness. Then (end day 0), quali-

fied subjects were exposed to RV by nasal administration of course drops containing 100–300 tissue culture infective doses (50%) of either RV39 or RV23. On each of the post-exposure days, the subjects followed the procedures done on the pre-exposure day with the exception of the pregnancy test. Approximately 28 days after virus exposure, convalescent blood was collected for testing of serum neutralizing antibody titer to the challenge strain of RV.

The investigators participating in the evaluations conducted at the cloister site were blinded to all psychological and biological measures as were the laboratories that conducted the assays. The subjects were paid \$800 for their participation. The protocol and informed consent were approved by the Institutional Review Boards at the Children's Hospital of Pittsburgh, the University of Pittsburgh and Carnegie Mellon University (Cohen et al., 2003).

2.1. Specific methods

Volunteers were interviewed over the phone on three evenings per week for 2 weeks during the month before quarantine and were interviewed on the first evening (before virus exposure) of quarantine. They were asked how accurately (0 = not at all accurate to 4 = extremely accurate) each of nine positive and nine negative adjectives described how they felt during the past 24 h. Positive adjectives represented three subcategories of positive emotion: vigor (lively, full-of-pep, energetic), well-being (happy, pleased, and cheerful), and calm (at ease, calm, and relaxed). Negative adjectives represented three subcategories of negative emotion: depression (sad, depressed, and unhappy), anxiety (on edge, nervous, and tense), and hostility (hostile, resentful, and angry). For each day, positive and negative mood scores were calculated by summing the ratings of the nine respective adjectives. The internal-reliabilities (Cronbach's α) for the seven assessments ranged between 0.89 and 0.93 for positive and between 0.87 and 0.92 for negative scores. Summary measures of PES and negative emotional style (NES) were created by averaging the daily mood scores (separately for positive and negative elements) across the 7 days (Cohen et al., 2003). In this population, the Pearson correlation coefficient between PES and NES scores was -0.39 .

On each day of quarantine, subjects were evaluated for symptoms and signs of illness as previously described (Cohen et al., 2003). By diary, they were asked to rate on a scale of 0 (none) to 4 (very severe) eight respiratory symptoms (congestion, runny nose, sneezing, cough, sore throat, malaise, headache, and chills) and four symptoms usually not associated with minor upper respiratory infections (dizziness, backache, faintness, and trembling hands) as experienced during the previous 24 h. For each symptom, the matched baseline score was

subtracted from each daily score (e.g., congestion day 1 minus congestion at baseline) to create an adjusted symptom score. Total (averaged across five post-challenge days) adjusted symptom scores were calculated separately for three clusters of vURI symptoms: nasal (congestion, rhinorrhea, and sneezing), throat (sore throat), and systemic symptoms (malaise, chills, headache, and cough). A similar score was calculated for a non-vURI symptom cluster (dizziness, backache, faintness, and trembling hands). A total adjusted vURI symptom score was also calculated that included the eight respiratory symptoms.

Daily secretion production was assessed by having the subjects collect all nasal secretions into pre-weighed tissues and seal the expended tissues in pre-weighed plastic bags. Secretion weight was calculated by subtracting the sum of the pre-weight values of tissue and bags from the corresponding weight measured at the end of the study day. A total secretion weight score was created by averaging the adjusted (minus baseline) weights over the five post-challenge days. Nasal mucociliary clearance function was assessed as the time required for a dyed saccharin solution administered into the anterior nose to reach the nasopharynx (maximum time allowed = 30 min). These data for individual subjects were excluded from the analysis if the baseline clearance time was ≥ 20 min. Total clearance score was calculated by averaging the adjusted (minus baseline) times for the post-challenge days.

The challenge viruses were safety tested, clinical RV isolates, passaged twice in human embryonic lung fibroblasts and supplied by Dr. Ronald Turner (University of Virginia, Charlottesville, VA). RV39- and RV23-specific serum neutralizing antibodies at screening and on approximately day 21 were assayed by a standard two-fold dilution method with titers reported as reciprocals of the final dilution. Antibody response to virus exposure was quantified as the ratio of the titer (maximum value = 32) at day 21 to that at the screening day and seroconversion was defined as a titer ratio of ≥ 4 (Gwaltney et al., 1989).

A sample of the nasal wash fluid recovered from each subject on each study day was immediately placed (3:1; v:v) in a cryovial containing 4 \times concentrated viral collecting broth and then frozen at -70°C . For RV detection, 0.2 ml of the mixture was inoculated into two tubes of human embryonic lung fibroblast cells and the cells were observed (14 days) for the development of a typical RV cytopathic effect. Data for subjects with viral recovery on study day 0 (pre-exposure) were excluded from further analyses. Subjects "shed virus" if the challenge-virus was detected during any of the five post-challenge days (Gwaltney et al., 1989).

An aliquot of the recovered wash fluid was frozen without dilution at -70°C and later assayed for IL-1 β , IL-6, and IL-8 protein using commercially available

ELISA kits (Endogen) and following the manufacturer's protocol (Gentile et al., 2003). Cytokine responses to exposure were quantified as the log area under the curve (AUC) of the five post-exposure days adjusted for baseline value.

3. Results

Because we were interested in symptom/sign expression among infected subjects, the analyses were limited to subjects who were infected by the standard clinical criteria—either shed virus or seroconverted (Gwaltney et al., 1989). Overall, 209 of 334 (63%) subjects shed virus and, of 209 subjects with pre-exposure antibody titers <8, 106 (51%) evidenced seroconversion (fourfold antibody increase). This resulted in 234 (70%) subjects classified as infected. The population demographics were 115 (49%) male; 160 (68%) white, 70 (30%) black, 4 (2%) other, with an age range of 18–54 years (28.7 ± 10.1 years). One hundred and ninety were infected with RV39 (81%) and 44 with RV23 (19%).

In the analyses, we used stepwise multiple linear regression and forced the standard control variables into the first step of the model equation. These variables were: challenge antibody titer (≤ 4 or ≥ 8), age (18–21, 22–32, 33–54 years), body mass index (weight [kg]/height [m]²), race (Caucasian, other), gender, virus-type (RV23 or RV39), month of exposure (March, May, July, Sep-

tember, or December), and education (\leq high school graduate, > than high school but < 2 years college, and ≥ 2 years college).

We began by assessing whether or not there were associations between each of the three cytokines and the disease outcomes. As apparent from Table 1, with one marginal exception (association of IL-8 and clearance score), greater nasal levels of all three cytokines were significantly associated with greater values for the measured vURI outcomes. In all cases, the IL-6 associations with outcomes were substantially larger (5.76–28.09% of variance) than the outcome associations with the other two cytokines (1.14–8.29% of variance). Moreover, the IL-6 level was most highly related to nasal signs (28.20% for mucus secretions) and symptoms (28.09%). As expected, IL-1 β and IL-8 levels were not associated with non-URI symptoms, but unexpectedly, IL-6 level was.

The first column in Table 2 presents the regression coefficients, percent explained variances, and probability levels for the contribution of PES to each of the measured outcomes after all controls were entered into the regression equation. Higher levels of PES were associated with fewer symptoms and signs. In a similar analysis, we found that NES predicted two of the seven outcomes, throat symptoms ($b = 1.00$, 2.6% variance, $p < .02$) and clearance score ($b = 0.14$, 2.2% of the variance, $p < .03$). For both, greater NES was associated with more severe illness. Because NES and PES are moderately negatively correlated, we assessed whether these

Table 1
Associations between the three cytokines and each outcome

	IL-1 β w/controls			IL-6 w/controls			IL-8 w/controls		
	<i>b</i>	% Var	<i>p</i>	<i>b</i>	% Var	<i>p</i>	<i>b</i>	% Var	<i>p</i>
Nasal symptoms	3.22 (± 0.74)	7.95	.000	7.09 (± 0.76)	28.09	.000	2.36 (± 0.53)	8.18	.000
Throat symptoms	0.86 (± 0.25)	5.15	.001	1.66 (± 0.28)	13.91	.000	0.50 (± 0.18)	3.39	.006
Systemic symptoms	2.43 (± 0.67)	5.66	.000	4.88 (± 0.73)	16.65	.000	1.76 (± 0.48)	5.76	.000
Non-URI symptoms	0.04 (± 0.43)	0.01	.926	1.43 (± 0.50)	6.50	.004	0.24 (± 0.32)	0.49	.439
Total symptom score	0.23 (± 0.05)	8.29	.000	0.43 (± 0.06)	22.18	.000	0.16 (± 0.04)	7.95	.000
Total secretion weight	0.22 (± 0.05)	6.60	.000	0.52 (± 0.06)	28.20	.000	0.15 (± 0.04)	5.81	.000
Clearance score	0.11 (± 0.04)	3.46	.005	0.16 (± 0.04)	5.76	.000	0.04 (± 0.03)	1.14	.112

Regressions included controls for age, season, gender, race, body mass index, virus, and pre-challenge virus-specific antibody.

Table 2
Positive emotional style (PES) associations with each of the outcome variables with and without IL-6 in the equation

	PES w/controls			PES w/controls and IL-6			
	<i>b</i>	% Var	<i>p</i>	<i>b</i>	% Var	% Change in effect	<i>p</i>
Nasal symptoms	-1.76 (± 0.64)	3.31	.006	-1.26 (± 0.57)	2.16	-34.76	.028
Throat symptoms	-0.67 (± 0.22)	4.16	.002	-0.55 (± 0.21)	3.13	-24.72	.008
Systemic symptoms	-1.21 (± 0.58)	1.99	.035	-0.87 (± 0.55)	1.12	-43.48	.116
Non-URI symptoms	-0.83 (± 0.40)	3.42	.040	-0.80 (± 0.38)	3.50	2.17	.038
Total symptom score	-0.17 (± 0.04)	6.10	.000	-0.14 (± 0.04)	4.97	-18.49	.001
Total secretion weight	-0.12 (± 0.05)	2.69	.014	-0.08 (± 0.04)	1.56	-41.91	.061
Clearance score	-0.15 (± 0.03)	8.94	.000	-0.14 (± 0.03)	7.95	-11.05	.000

Percent change in effect is the reduction in the effect size of PES when IL-6 is added to the equation. Regressions also include controls for age, season, gender, race, body mass index, virus, and pre-challenge viral-specific antibody.

associations were independent by entering both PES and NES into the second step of the equations. There, the NES associations were no longer significant ($p > .28$ and $p > .98$, respectively), while the PES associations were retained ($b = -0.55$, 2.1%, $p < .03$ for throat symptoms; $b = -0.16$, 6.7%, $p < .01$ for clearance time).

We also analyzed the association between PES and each of the three cytokine responses. The only documented association was a marginal relationship between PES and the logged AUC of IL-6 ($b = -0.08$, $p = .12$). Fig. 1 shows the averages and respective standard errors of the adjusted IL-6 levels as a function of time for groups defined by PES tertiles. The greatest IL-6 level and largest differences among groups occurred on the third day post-challenge with lesser differences on other days. To get a more sensitive assessment of the day-to-day changes in IL-6 in relation to PES, we conducted a repeated measures analysis of covariance using the standard covariates and individual logged adjusted IL-6 levels on each day (instead of AUC) as the dependent variables. This analysis lost 16 subjects who were missing an IL-6 value on 1 day of the trial. That analysis identified both a main effect of PES ($F[2,205] = 4.8$, $p < .05$) and a marginal PES-by-day interaction ($F[8,205] = 2.0$, $p = .059$).

Finally, we fit a series of models wherein IL-1 β , IL-6, and IL-8 were each added to the first step of the equations that predicted each outcome from PES score. The reduction in explained variance after adding a specific cytokine provides an estimate of the extent to which that cytokine may operate to mediate the association. The percentage reduction for each included cytokine is presented in Table 2.

As expected (since they were not associated with PES), neither IL-1 β nor IL-8 reduced any PES effect by more than 3%. In contrast, with the exception of the control (non-URI) symptoms, inclusion of IL-6 levels substantially reduced the effect size for PES on all of the outcome measures (reductions ranging from 11 to 43% of the effect).

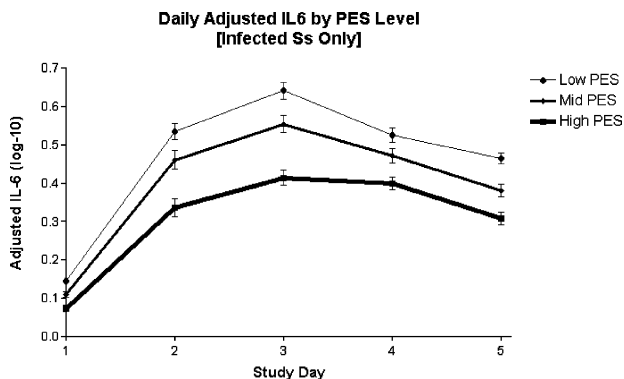


Fig. 1. Average adjusted IL-6 levels and associated standard errors as a function of study day for three groups defined by PES tertile.

4. Discussion

In an earlier article, we reported that greater levels of PES predicted a lesser risk for objectively defined colds in persons experimentally infected with RV. While PES could promote resistance to illness by encouraging health-enhancing behaviors, building resources to cope with stress and enhancing regulation of emotion-sensitive biological systems (Fredrickson, 2004; Pressman and Cohen, in press), none of these pathways examined at that time explained the observed relationship (Cohen et al., 2003). Consequently, in this report we focused on the newly available data for three assayed cytokines as potential linkage mediators and explored the potential specificity of PES-cytokine associations for specific components of illness expression.

Consistent with our previous results for cold incidence, we found that PES was associated with a lesser expression of a wide range of symptoms and signs of RV infection. PES was not only associated with fewer total symptoms, but also with all three of the vURI symptom clusters, although the effect size was greatest for throat symptoms, followed closely by nasal symptoms and lastly systemic symptoms. For measured signs, PES had a larger association with clearance function—an objective indicator of congestion, and a smaller (one-third the effect) association with secretion weights—an objective indicator of mucus production. In sum, PES was most strongly associated with nasal and throat symptoms and signs, and somewhat less so with systemic symptoms. Interestingly, PES was also associated with fewer non-vURI symptoms. This may be explained by past evidence showing that high PES results in lesser symptom reporting even when objective illness is held constant (Cohen et al., 2003).

Our analyses of the three cytokines for a possible role in linking PES to symptom/sign expression identified only IL-6 as a reasonable candidate. Specifically, higher PES was associated with lower IL-6 levels and lesser symptom/sign responses. Lower IL-6 levels were also associated with lesser symptom/sign responses, and controlling for IL-6 levels decreased the effect of PES on vURI symptoms/signs by 25–44%. The IL-6 mediation hypothesis is best supported for the associations between PES and systemic and nasal symptom clusters and between PES and total secretion weight. There was no evidence that the two other assayed cytokines contributed to the IL-6 mediation of PES on any of the symptom/sign clusters. Interestingly, there was no similar evidence that IL-6 mediated the association between PES and non-URI symptoms. This last result supports the specificity of IL-6 to illness expressions directly related to the infection. This observation is also consistent with the above argument that the association of PES and non-URI symptoms is driven by reporting bias, not underlying pathology.

In sum, higher PES is associated with fewer signs and symptoms of disease in subjects experimentally infected with RV. The mediational analyses support the hypothesis that PES influences illness expression through its impact on IL-6 production. This is consistent with our earlier report that IL-6 mediated the association between psychological stress and illness expression during influenza infection (Cohen et al., 1999) and demonstrates the generality of this result to explaining the effect of another psychological characteristic (PES) on illness expression that is in response to a different virus (rhinovirus). However, this interpretation of the data is presented with a number of caveats. First, because the test of mediation is correlational, it is possible that assumed cause–effect directionalities are not correct, with, for example, the rises in IL-6 resulting from illness as opposed to causing illness. There, PES may moderate illness via an alternative mechanism with the consequent relationship between PES and IL-6 being mediated by the effect of PES on illness. While numerous past studies of causality consistently link IL-6 to illness expression during a vURI, this possibility remains viable until specific IL-6 modulators are developed and shown to affect illness expression (Doyle et al., 2005). Second, the three cytokines assayed are not inclusive of those produced during a vURI and it is possible that the “true” linking cytokine (or other chemical) was not assayed but exerts effects on both IL-6 and symptom/signs, independently. Third, it is possible that PES actually influenced the extent of infection and that the greater the replication of virus, the greater the release of IL-6 and expression of symptoms. Finally, associations between PES and symptom expression were small calling into question the clinical significance of this psychological modifier to illness. However, these associations were estimated after entering the seven control variables and this conservative technique attributes any shared explained variance to the control variables. Moreover, all outcome variables were summary representations for the 5-day-observation period, when in fact, the period of illness was limited to 2 or possibly 3 days. This results in the addition of error variances to the measure which may cause an underestimation of the true effect sizes.

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