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Infection-induced proinflammatory cytokines are associated with decreases in positive affect, but not increases in negative affect

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Abstract

Infection commonly triggers nonspecific psychological and behavioral changes including fatigue and malaise, anhedonia, inability to concentrate, and disturbed sleep that collectively are termed "sickness behaviors". Converging evidence from several lines of research implicate the activities of proinflammatory cytokines as a cause of sickness behaviors. Here we elaborate upon the findings of previous research by examining whether infection-associated elevations in local proinflammatory cytokines are associated with increased negative mood and decreased positive mood. One hundred and eighty-nine healthy adults were experimentally exposed to rhinovirus or influenza virus during a 6-day period of quarantine. Infection, objective signs of illness, nasal IL-1β, IL-6, and TNF-α, and self-reported affect were assessed at baseline and on each of the five post-challenge quarantine days. In the 153 persons who became infected following exposure to the challenge virus, daily production of IL-6, but not IL-1β or TNF-α, was associated with reduced concurrent daily positive affect. One-day lagged analyses showed that daily production of all three cytokines was related to lower positive affect on the next day. All lagged associations were independent of previous-day positive affect and objective signs of illness (mucus production, mucociliary clearance function). There were no associations between cytokines and negative affect. Findings support a causal association between pathogen-induced local cytokine production and changes in positive affect over a 24-h timeline.
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1. Introduction

Infection commonly triggers nonspecific psychological and behavioral changes that collectively are termed "sickness behaviors" (Kent et al., 1992). Changes include experiences of fatigue and malaise, loss of interest in pleasurable activities such as eating and socializing, inability to concentrate, and disturbed sleep. Once thought merely to be a reactive response to the discomfort and debilitation of physical illness, sickness behavior now is suggested to play an integral role in the body's protective response to infection (Hart, 1988).

Converging evidence from several lines of research implicates the activities of proinflammatory cytokines as a cause of sickness behaviors (Kelley et al., 2003). For example, intraperitoneal injection of exogenous cytokines to laboratory rats produces behavioral changes consistent with sickness behavior such as suppressed food consumption (Sammut et al., 2001) and decreased social exploration (Bluthé et al., 1994). Similarly, research on human cancer patient populations showed that cytokine treatment is associated with sickness behaviors such as increased fatigue

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(Eskander et al., 1997; Rinehart et al., 1997), depressed mood (Capuron et al., 2001), and anorexia (Schiller et al., 1991).

Though suggestive of a role for cytokines in eliciting sickness behavior, the above studies are limited in that they may not accurately reflect proinflammatory processes initiated by a typical illness episode. An important limitation of the animal studies is that concentrations of injected cytokines far exceed cytokine levels that typically circulate during a chronic inflammatory reaction (Pollmacher et al., 2002). A similar argument can be made with regard to cytokine therapy, where dosages of administered cytokines often exceed physiologic levels typically associated with response to injury or infection.

Two recent studies examined the relation of cytokines to psychological and behavioral changes following exposure to mild pathogenic stimuli. In the first study, plasma cytokine levels, physical symptoms, sickness behavior, and psychological state were assessed 1, 3, and 9 h after intravenous administration of *Salmonella abortus equi* endotoxin or saline (Reichenberg et al., 2001). As expected, exposure to endotoxin, but not placebo, was associated with a significant increase in plasma IL-6 and TNF-α within 4 h, and with increased anxiety and depressed mood. Endotoxin-induced enhancement of anxiety and depressed mood showed a significant temporal correlation with observed increases in both proinflammatory cytokines. In a later study (Wright et al., 2005), administration of *S. typhi* vaccine, but not placebo, was associated with increased IL-6 concentrations, increased negative mood, and decreased positive mood within the first 3 h post-injection. Post-vaccination negative mood, but not positive mood, was correlated with the increase in IL-6.

In the present report, we elaborate upon these findings by testing the hypothesis that elevations in local (site of infection) cytokine production consequent to experimental infection with a viral upper respiratory pathogen (vURI) will be associated with alterations in self-reported affect. Though instantaneous reports of positive and negative affect tend to be strongly inversely correlated, there are circumstances under which individuals' experiences of positive and negative emotions can be partly independent of one another (e.g., Diener and Emmons, 1985; Watson et al., 1988). For example, when reflecting on the last day or week, one reasonably might report having been both happy and sad. There is also evidence that activation of separate cortical pathways may give rise to the experience of positive and negative emotion, with the former being related to the "approach" system and the latter to the "withdraw" system (Davidson, 1998). Given the experiential and proposed physiological independence of positive and negative affect, it is possible that proinflammatory cytokines relate differentially to positive and negative mood states. Reduced positive affect, or anhedonia, may be a direct effect of proinflammatory cytokine activity because consequent suppression in reward-seeking behaviors would allow energy to be shifted toward metabolic processes involved in fighting off infection. By comparison, elevated negative affect may be an indirect consequence of proinflammatory cytokine activity that arises as a response to the experience of cytokine-induced physical symptoms. This suggests that elevated local (nasal mucus) cytokines subsequent to viral infection will be associated with (1) increases in negative affect (NA) and (2) decreases in positive affect (PA), and that these associations occur for different reasons.

In separate models, we examined whether daily local (nasal) cytokine levels measured during experimental Influenza A virus or Rhinovirus 39 infections of adult humans predict increased NA and decreased PA on the same day and on the following day, and determined if any or all of the observed effects were independent of objective vURI signs (mucus weight and mucociliary clearance function). We included data from two different virus types to show that the association between cytokines and affect is consistent across virus types.

2. Method

We report a secondary analysis based on a large clinical trial designed to assess psychosocial predictors of resistance to viral infection. The design of the study involved an experimental model of upper respiratory virus infection that was employed by the second and third authors in a previous study of trait positive emotional style, average nasal cytokine levels, and illness expression (Doyle et al., 2006). Subjects were 95 men and 98 women, aged 21–55 years (mean age = 37.3, SD = 8.8), studied between 2000 and 2004 and experimentally exposed to one of two safety-tested upper respiratory viruses, Influenza A and rhinovirus 39 (RV39). Participants were recruited by newspaper advertisements soliciting subjects for an experimental study of the psychosocial risk factors that moderate viral upper respiratory infection and illness. The study was conducted in 11 cohorts of between 2 and 39 participants each.

Of the 193 subjects, 108 (56%) were white, 72 (37%) were black, and 13 (7%) indicated other racial/ethnic categories. The mean education was 13.8 years (SD = 2.2), 47% of subjects were smokers, and mean body mass index (BMI) was 29.0 (SD = 7.1). The study protocol was approved by the IRBs at the University of Pittsburgh and Carnegie Mellon University, all subjects provided written informed consent and were paid $820 for completing the study.

Potential subjects were screened by telephone and later interviewed and examined by a physician. To maximize the rate of infection, only subjects with an antibody titer to the challenge virus of $\leqslant 4$ were included. Subjects were excluded if they had a chronic medical condition, were not in generally good health, were pregnant or lactating, or had a recent or current upper respiratory infection. Individuals also were excluded if regularly taking medication, with exception of birth control, hormone replacement therapy, analgesics, and topical eczema/psoriasis medications.

Eligible participants who were exposed to RV39 were quarantined in a hotel for 1 day before and 5 days after viral challenge. Those who were exposed to Influenza A virus were quarantined for 1 day before and 6 days after challenge. To maintain comparability, we excluded the sixth day after challenge for those exposed to the Influenza A virus.

On each day of quarantine subjects received a general physical exam and a nasal wash with recovered fluids submitted for cytokine assay and virus culture. Subjects also were examined for objective signs of illness (mucus production and mucociliary clearance function; see Doyle et al., 2006), and completed questionnaire diaries that assessed subjective vURI symptoms (not discussed here), health practices, and moods. On the first day of quarantine and before virus exposure, female subjects received a pregnancy test. About 21 days post-quarantine subjects had blood drawn for assay of convalescent antibodies to the challenge virus.

2.1. Virus exposure

At the end of the first day of quarantine (5:00 p.m.), participants were given nasal drops containing 125 tissue culture infectious dose$_{50}$ ($TCID_{50}$) of RV39 [$n = 155$] or 10^5 $TCID_{50}$ of influenza A/Texas/36/91 [$n = 38$]. Only one virus was used in a cohort trial.

2.2. Daily affect

At about 1:30 p.m. on each day of quarantine, subjects completed a questionnaire that assessed daily affect. Questionnaires included six positive and six negative adjectives for which subjects rated how they had been feeling since awakening that morning (Cohen et al., 2003). The positive scale assessed vigor (*full of pep*, *lively*), calm (*at ease*, *calm*), and well-being (*cheerful*, *happy*). The negative scale assessed depression (*sad*, *unhappy*), anxiety (*on edge*, *tense*), and anger (*angry*, *hostile*). Responses were scored on a five-point analog scale (range: 0 = *you haven't felt that way at all today* to 4 = *you felt that way a lot today*). Daily PA was calculated by summing the ratings of the six positive adjectives; and daily NA was calculated by summing the ratings of the six negative adjectives. The internal reliabilities (Cronbach's α) for each daily assessment ranged from .85 to .90 for PA and .71 to .89 for NA.

2.3. Viral cultures and antibody response

Virus-specific neutralizing antibody titer was measured in serum collected before and 28 days after virus exposure (Gwaltney et al., 1989). An aliquot of the nasal lavage sample from each study day was mixed with viral transport medium, frozen at −70 °C and later cultured for RV39 or influenza virus using standard techniques (Gwaltney et al., 1989; Tobita et al., 1975). Subjects were classified as infected if challenge virus was isolated in nasal washings on any of the post-exposure study days or if there was at least a 4-fold increase in virus-specific antibody titers (Doyle et al., 2006). Data from subjects for whom the viral culture was positive before virus exposure were eliminated from analyses.

2.4. Cytokine analysis

Nasal wash fluid was assayed for cytokine protein using the BioSource Ten-plex Bead Immunoassay and methods provided by the manufacturer (BioSource International, Camarillo, CA). Because the Ten-plex allows simultaneous measurement of multiple biological markers in a single sample, all three cytokines were assayed at once. Antibodies to the cytokines of interest (IL-6, IL-1β, or TNF-α) were covalently linked to beads (microspheres) with a unique fluorophore. Samples were incubated with antibody-coated beads in a 96-well filter plate, washed, and then incubated with a biotinylated antibody specific for a different binding site on the same analyte. After washing to remove excess biotinylated antibody, samples were incubated with streptavidin-RPE (fluorescent), which binds to biotinylated antibody for detection. Samples were washed to remove unbound dye, and bead-bound fluorescence was quantified using the Bio-Plex system (Bio-Rad Laboratories, Inc., Hercules, CA). The intensity of fluorescence was measured and converted to the concentration of each cytokine. Cytokine data were included in the present analyses only if they were complete for each study day. Data from four subjects were excluded due to missing entries for one or more of the measured cytokines on one or more study days. Thus, the final sample included in analyses was $n = 189$.

2.5. Standard control variables

When analyzing mean differences in post-exposure cytokine production between subjects who did and did not become infected with the challenge virus, we controlled for virus, pre-challenge antibody titer (within virus), age, education, body BMI (weight [kg]/height [m]^2), race (white, black or other minority), sex, and season of exposure (spring, summer, autumn, and winter).

3. Statistical analysis

The SAS system for Windows (release 8.02, SAS Institute, 1995) was used to perform all statistical analyses. We used *t*-tests to examine differences between infected and noninfected persons on demographic variables, smoking status, BMI, affect, cytokine production, and signs of illness. As daily NA, mucus production, mucociliary clearance, and cytokine variables all were positively skewed, a log transformation was applied to these data prior to conducting mean comparisons. Prior to conducting the *t*-tests, we used the "folded" *F*-statistic (F') to examine the data for equality of variances. If Prob $F' < .05$, we reported the *t*-statistic for unequal variances (Satterthwaite's approximate *t*-statistic) and the corresponding adjusted degrees of freedom.

We used multilevel modeling (Raudenbush and Bryk, 2002) to examine the association of cytokine production with concurrent affect. Two features of this technique render it appropriate for examining the present hypotheses. First, multilevel modeling is more powerful than repeated measures analysis of variance for examining within-person associations because multilevel methods allow users to control for the tendency of measures that are taken closer together in time to correlate more highly than measures taken farther apart. Second, multilevel models can be designed to conduct lagged analyses wherein outcomes are predicted from variables measured at an earlier time (e.g., IL-6 level today predicting tomorrow's affect). An advantage of lagged analyses is that they allow one to covary the value of the outcome at the time of prediction, thus resulting in an analysis of whether the predictor is associated with a change in the outcome over a specific time period. We used SAS PROC MIXED to conduct all multilevel analyses.

4. Results

Infection was not documented in 36 (19%) of the 189 included subjects who were exposed to challenge virus (virus was not isolated in nasal washings on any of the post-exposure study days and a ⩾4-fold increase in virus-specific antibody was not detected on convalescent assay). To determine if observed effects were specific to infection-induced elevations in cytokine production, we examined infected and uninfected groups separately. Table 1 reports baseline characteristics of the population separately for

Table 1
Sample descriptives

	Infected $n = 153$	Uninfected $n = 36$
Age	37.3	37.1
% female	49.7	55.6
% nonwhite	44.4	41.7
% current smokers	45.1	55.6
BMI	29.8	30.7

Table 2
Pre- and post-exposure nasal mucus cytokine and affect levels

	Pre-exposure			Post-exposure		
	Infected	Uninfected	*p* (difference)	Infected	Uninfected	*p* (difference)
IL-6 (pg/mL)	58.86 (127.61)	40.52 (69.26)	.96	162.97 (376.24)	41.67 (51.32)	<.01
IL-1β (pg/mL)	135.17 (297.33)	67.68 (123.77)	.27	151.33 (267.19)	87.97 (96.87)	.89
TNF-α (pg/mL)	237.89 (580.10)	111.49 (228.32)	.43	356.34 (844.10)	164.15 (249.57)	.59
Positive affect	13.22 (5.62)	10.56 (3.82)	<.01	12.81 (5.57)	9.82 (3.72)	.01
Negative affect	1.51 (2.74)	.56 (1.25)	<.01	1.71 (2.43)	.91 (1.34)	<.01

these subgroups. Subgroups did not differ on demographic variables, BMI or percentage of current smokers.

Table 2 reports average pre- and post-exposure cytokine levels and affect scores. Persons who became infected following exposure to viral challenge reported higher average pre-exposure PA and NA compared to those who did not become infected. Infected persons also showed higher average pre-exposure cytokine levels than their uninfected counterparts, but these differences were not statistically significant. After viral challenge, infected persons again reported higher average PA and NA relative to uninfected persons. On average, infected persons also produced more IL-6 post-exposure than uninfected persons, but between-group differences in IL-1β and TNF-α were not significant.

Following exposure to challenge, infected persons produced more mucus (mean = 3.80 g vs. 0.72 g; $F' = 4.90$, $p < .001$; $t(125) = 4.86$, $p < .001$) than their uninfected counterparts, but the groups did not differ significantly with regard to mucociliary clearance time (mean = 3.72 min vs. 2.65 min; $F' = 1.06$, $p = .88$; $t(187) = .91$, $p = .37$).

4.1. Concurrent association between daily cytokine levels and positive and negative affect

Table 3 reports results of regression models for the association between daily cytokine levels and concurrent daily affect in subjects who did and did not become infected after viral exposure. As these models assessed within- rather than between-person effects, controls for between-person influences (e.g., age, gender, BMI) were not included. However, because of the significant between-subgroup difference in affect, we included baseline affect as a covariate in the model. Results showed that infected persons reported less PA on days characterized by greater IL-6 production but that PA was not related to concurrent production of IL-1β or TNF-α. By comparison, daily NA was unrelated to concurrent daily cytokine production in the infected subgroup. Cytokine levels were not related to concurrent affect in the uninfected subgroup.

Table 3
Results of regression models of the association between log-transformed daily cytokine production and concurrent affect

	Infected			Uninfected		
	b	*t*	*p*	*b*	*t*	*p*
Positive affect						
IL-6	−.50	−3.81	<.001	−.26	−.98	.33
IL-1β	.02	.16	.87	.01	.05	.96
TNF-α	−.16	−1.35	.18	−.07	−.37	.71
Negative affect (log-transformed)						
IL-6	.03	1.19	.23	.02	.37	.72
IL-1β	.01	.46	.65	.04	.94	.35
TNF-α	.02	.96	.34	.05	1.34	.18

Control for baseline affect.

As there is no association of cytokine levels and NA, there is no support here for our argument that proinflammatory cytokines may influence NA through their effects on illness. However, it is possible that the daily declines in PA associated with infection-induced increases in IL-6 production might arise as a psychological response to the experience of having a cold rather than as a direct consequence of elevated cytokines, per se. Indeed, among the infected subgroup, PA was lower on days characterized by greater mucus production ($b = -2.04$, $t(150) = -6.13$, $p < .001$) and marginally lower on days with reduced mucociliary clearance function ($b = -.52$, $t(151) = -1.78$, $p < .10$). However, concurrent IL-6 remained an independent predictor of daily PA in the infected subgroup after simultaneous control for baseline PA and the two objective indicators of illness severity ($b = -.32$, $t(150) = -2.32$, $p < .05$).

4.2. Lagged association between daily cytokine levels and positive and negative affect

To determine if infection-provoked changes in daily cytokine production precede changes in daily affect, we examined, in separate models, whether cytokine production was associated with affect on the following day. For these analyses, we created 1-day lag variables from the cytokine data and regressed daily affect on the previous day's cytokine levels. To control for the influence of previous-day affect, we computed 1-day lag affect variables and included either lagged PA or NA in the model. As indicated by Table 4, higher IL-6, IL-1β, and TNF-α production in the infected subgroup was associated with reports of lower PA on the next day, independent of previous-day PA. For the uninfected subgroup, cytokines were not related to affect on the next day. Because daily mucus production and mucociliary clearance were related to concurrent reports of lower PA, we conducted analyses among infected persons that included additional controls for objective signs of illness on the same day. Results of these analyses are displayed in Table 5 and show that each of the three cytokines remained independent predictors of PA on the following day. To rule

Table 4
Results of regression models of the association between log-transformed daily cytokine production and affect on the following day

	Infected			Uninfected		
	b	*t*	*p*	*b*	*t*	*p*
Positive affect						
Previous day IL-6	−.48	−3.54	< .001	−.27	−1.08	.28
Previous day IL-1β	−.39	−2.72	< .01	−.36	−1.58	.12
Previous day TNF-α	−.35	−2.90	< .01	−.23	−1.13	.26
Negative affect						
Previous day IL-6	−.005	−.23	.82	−.01	.17	.86
Previous day IL-1β	−.01	−.64	.52	−.01	−.23	.82
Previous day TNF-α	.002	.12	.91	−.02	−.52	.60

Control for previous-day affect.

Table 5
Results of analyses predicting daily positive affect from log-transformed cytokine production on the previous day

	b	*t*	*p*
Previous day IL-6	−.31	−2.24	<.03
Previous day IL-1β	−.35	−2.45	<.02
Previous day TNF-α	−.29	−2.38	<.02

Control for same day mucus production (log-transformed) and mucociliary clearance (log-transformed) and previous-day positive affect.

out the possibility that changes in NA or PA might be driving changes in cytokines on the next day, we conducted analyses wherein affect predicted next-day cytokine production. None of these analyses produced significant results (p's > .05).

As low scores on the *vigor* component of the PA scale might indicate sickness-induced malaise, we separately examined the two subcomponents of PA that did not contain adjectives that could be interpreted as markers of malaise—*well-being* and *calm*. Results of these analyses approximated those performed using the entire PA scale. For example, for IL-6, the lagged multivariate association with *well-being* on the following day was significant ($b = -.15$, $t = -2.61$, $p < .01$) and the association with "calm" approached significance ($b = -.11$, $t = -1.88$, $p = .06$).

4.3. Moderating effect of challenge virus type

Because RV39 and influenza virus have distinct pathogenic mechanisms for inducing infection and illness (Subauste et al., 1995), we examined whether the significant associations reported above were moderated by virus type. For no model was the interaction between cytokine production and virus type significant (p's > .10).

5. Discussion

In an earlier paper, we report an association between trait positive emotional style and reduced average cytokine production across 7 days following intranasal injection with rhinovirus (Doyle et al., 2006). Here, we report the association of daily fluctuations in post-exposure cytokines with changes in state positive and negative mood. Among subjects who became infected following exposure to the challenge virus, daily production of nasal mucus IL-6, but not IL-1β or TNF-α, was associated with reduced concurrent daily PA. One-day lagged analyses showed that daily production of all three cytokines was related to lower PA on the following day. By comparison, there were no associations between cytokines and NA. Thus, the hypothesis that elevated proinflammatory cytokines result in more severe illness signs and that illness signs, in turn, induce greater NA is not supported here. The present findings might be compared to those of two previous studies that examined changes in cytokine production and mood after exposure to endotoxin. In contrast to the results reported here, Wright and colleagues found that negative mood, but not positive mood, was associated with endotoxin-induced IL-6 production (Wright et al., 2005). Similarly, Reichenburg and colleagues found that endotoxin-induced elevations in plasma IL-6 and TNF-α were related to two measures of negative mood: depressed mood and anxiety (Reichenberg et al., 2001). These authors, however, did not examine the relation of cytokines to positive mood.

There are several explanations for the discrepancy between studies regarding associations of cytokines with affect. First, we tested post-exposure changes in cytokines and affect over a full day while the two earlier studies tested changes in cytokines and mood over several hours. It is possible that short-term vs. long-term elevations in cytokine production yield differing effects on mood. Alternatively, the lag time needed for cytokine activities to be translated into mood effects may differ for NA and PA. Our data suggest that a relatively long (up to 1 day) exposure and or lag times may be required to observe effects of cytokines on PA. Second, it is possible that cytokine levels induced by viral infection were much lower than those induced by an acute challenge with bacterial products. Bacterial product challenges are known to greatly increase systemic cytokine levels (Krabbe et al., 2005; Schinkel et al., 2005). Finally, a multitude of host-produced chemicals exhibit potent immunomodulatory effects (e.g., cytokines, chemokines, histamine, leukotrienes, and bradykinin) and few of these have been assayed in any given disease model (Doyle et al., 2005). Thus, the profile of cytokines and other biologically active host-derived chemicals (both measured and nonmeasured) may be quite different for the two model stimuli.

It is of interest that higher nasal IL-6 production consequent to a vURI coincided with concurrent reports of lower PA, but this was not true for IL-1β or TNF-α. However, nasal levels of all three cytokines predicted PA on the next day. This phase delay between nasal cytokines and PA might be explained by the need for locally produced (nasal mucosa) cytokines to accumulate in the circulation in order to influence mood. Local cytokine production can raise serum levels either via spillover into the blood or by induction of down-stream cytokine production in the systemic circulation. Few studies have concurrently measured nasal

and blood cytokines over the course of infectious illness. However, two studies of vURIs secondary to influenza A infection (Hayden et al., 1998; Kaiser et al., 2001), and one study of vURI secondary to infection with RV16 (Jarjour et al., 2000) reported temporally phased increases in nasal and serum cytokines. The similarity of serum and nasal secretion measures is supported by the latter study's reported correlation of .95 between nasal mucus and plasma levels of granulocyte colony stimulating factor (G-CSF) (Jarjour et al., 2000).

Several mechanisms have been proposed to explain associations between peripheral cytokines and psychological function (Kelley et al., 2003; Wichers and Maes, 2002). These include transport of peripheral cytokines across the blood–brain barrier (Banks et al., 2001), activation of the hypothalamic–pituitary–adrenal (HPA) axis via stimulation of the afferent vagus nerve (Maier et al., 1998), and inhibition of the 5-HT pathway (Myint and Kim, 2003). Proposed secondary mediators include prostaglandin E2 (Avitsur et al., 1999) and indoleamine-2,3-dioxygenase (Myint and Kim, 2003). Given these suggested mechanistic pathways and the present observations, we propose the following pathway for the effect of vURI on mood: local nasal cytokine production subsequent to infection with a nasal mucosal virus leads to increased systemic cytokine levels by spillover or induced production in the circulation. Elevated circulating cytokines then induce production of secondary mediators with central effects that ultimately influence mood. Because serum cytokine levels were not measured here, additional work is needed to test this hypothesis.

An alternative interpretation of the present findings is that the lagged associations between cytokines and PA reflect the association between proinflammatory proteins and sickness-induced malaise because low scores on the *vigor* component of the PA measure (*lively*, *full-of-pep*) could indicate physical fatigue rather than low psychological affect. To address this issue, we separately examined the two subcomponents of PA that did not contain adjectives that could be interpreted as markers of malaise—well-being and calm. Even with the modest variability associated with the shorter scales, results of these analyses were largely similar to the findings reported for the intact scale. Thus, we can rule out the possibility that associations between cytokines and PA score on the following day were driven by sickness-related malaise.

There are possible clinical implications of the present findings. Co-morbidity of physical disease and mental depression is well recognized (Katon and Sulivan, 1990; Katon, 2003). For example, rates of major depression among HIV+ women far exceed the rates among HIV− women (Morrison et al., 2002). Similarly, the rates of major depression and adjustment disorder among adult cancer inpatients have been estimated to range from 23 to 60% (Newport and Nemeroff, 1998). Low PA, in addition to high NA, is fundamental to the definition of clinical depression (Watson et al., 1988). Thus, in light of the present findings, it is possible that serious and chronic infectious diseases such as AIDS and infection-triggered cancers may directly (as well as indirectly) influence clinical depression through their effects on PA.

It should be noted that although detected associations were significant only for subjects who became infected, in some cases effect sizes were comparable for the infected and uninfected subgroups. For example, the effect size for the lagged association of IL-1β with PA was similar for the infected and the smaller (and hence less powered) uninfected subgroups ($b = .39$ and $b = .36$, respectively). It is possible that our method of assessing infectious status (isolation of challenge virus from nasal secretions or seroconversion) was not sufficiently sensitive to identify all persons with sufficient viral replication to trigger cytokine production, thus contaminating the uninfected group with false negatives. More sensitive techniques, such as polymerase chain reaction (PCR), for example, may have more accurately identified infected subjects.

A limitation of the present study is that cytokines were measured only in nasal secretions and not in plasma. It is plasma proinflammatory cytokines that have been implicated in behavior changes such as fatigue and cognitive dysfunction (e.g., Dantzer, 2001). Also, several cytokines that were not examined in the present study have been implicated in the production of sickness behaviors. Intravenous administration of IL-2 to cancer patients, for example, is associated with development of depression (Capuron et al., 2001). Inclusion of additional cytokines, from nasal secretion and plasma sources, would have strengthened the findings reported here.

In summary, the present findings add support to the proposed contribution of proinflammatory cytokines to the development of sickness behaviors, in particular anhedonia. In lag analyses that controlled for same-day illness severity (mucus production and mucociliary clearance), we were able to predict changes in affect from cytokines on the previous day. Given the temporal precedence of the cytokine measures in these analyses, we can interpret our findings as suggesting that (a) elevations in local (nasal) cytokines consequent to vURI precede changes in affect and (b) the present results cannot be explained by reverse causation, i.e., that PA influenced changes in cytokine production on the following day. Thus, our data support a potential causal association between pathogen-induced local cytokine production and changes in affect over a long (24 h or more) timeline.

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