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Production of pro-inflammatory cytokines correlates with the symptoms of acute sickness behaviour in humans

U. VOLLMER-CONNA*, C. FAZOU, B. CAMERON, H. LI, C. BRENNAN, L. LUCK, T. DAVENPORT, D. WAKEFIELD, I. HICKIE AND A. LLOYD

School of Psychiatry, University of New South Wales, 2052, Australia; Inflammation Research Unit, School of Medical Sciences, University of New South Wales, 2052, Australia; Brain and Mind Research Institute, The University of Sydney, Sydney, New South Wales, Australia; Beyondblue: the National Depression Initiative, Melbourne, VIC, Australia

ABSTRACT

Background. Elaboration of the concept of cytokine-induced sickness behaviour in recent years has opened new avenues for understanding brain involvement in sickness and recovery processes. Additionally, this has led to much speculation about the role of the immune system in neuropsychiatric syndromes, including depression and chronic fatigue. However, few studies have examined this phenomenon as it naturally occurs in sick humans, and none has attempted to document the quantitative relationships between cytokine levels and non-specific symptoms. The aim of this research was to examine human sickness behaviour and its immunological correlates in documented Epstein–Barr virus (EBV), Q fever or Ross River virus (RRV) infections.

Method. We studied two separate samples. The first consisted of 21 patients with acute Q fever. The second included 48 patients with acute RRV or EBV infection. Psychological and somatic symptom profiles were derived from self-report measures completed at enrolment. Quantification of pro-inflammatory cytokines [interleukin (IL)-1β and IL-6] in sera and supernatants of peripheral blood mononuclear cell (PBMC) cultures was undertaken by specific ELISAs.

Results. Levels of IL-1β and IL-6 spontaneously released from PBMC cultures were consistently correlated with reported manifestations of acute sickness behaviour including fever, malaise, pain, fatigue, mood and poor concentration.

Conclusions. IL-1β and IL-6 produced as part of the host response represent sensitive markers of sickness behaviour in humans with acute infection. Further work is needed to systematically characterize the spectrum and natural history of sickness behaviour in humans and to elucidate its biological basis.

INTRODUCTION

Acute infective illness is typically accompanied by a cluster of non-specific symptoms including fever, an increased need to sleep, hyperalgesia, anorexia, loss of interest in usual activities, social interaction and body care, depressed mood and impaired concentration (Hart, 1988; Dantzer *et al.* 1996; Konsman *et al.* 2002). Despite being the major determinant of short-term disability, these symptoms are commonly dismissed or relatively ignored by physicians because they represent a prevalent and universal illness experience. Research over the past decade has suggested that these psychological and behavioural changes associated with acute illness, termed 'sickness behaviour', are not the undesirable side effects of the host response to

* Address for correspondence: Dr Uté Vollmer-Conna, Department of Human Behaviour, School of Psychiatry, University of New South Wales, Sydney 2052, Australia.
(Email: ute@unsw.edu.au)

pathogens, but rather constitute a highly organized, evolved disease-fighting strategy that functions to complement the immune response (Vollmer-Conna, 2001).

An accumulation of evidence suggests that sickness behaviour is triggered by the activities of pro-inflammatory cytokines, notably interleukin (IL)-1β, IL-6 and tumour necrosis factor alpha (TNF-α) produced by activated immune cells (notably monocyte/macrophages and lymphocytes). These cytokines act as messenger molecules and play a pivotal role in the orchestration of the acute phase response and pathogen clearance (Dinarello, 1997). Moreover, we now know that the necessary synchrony between metabolic, physiological and behavioural aspects of the individual's response to infection depends on the action of these same cytokines (Papanicolaou *et al.* 1998; Konsman *et al.* 2002). Several mechanisms have been identified that facilitate the transmission of peripheral cytokine signals to central nervous system (CNS) targets. These include direct neural pathways via primary autonomic (vagal) afferents (Goehler *et al.* 1999; Maier & Watkins, 1999), and humoral mechanisms involving cytokine entry at brain regions where the blood–brain barrier is weak or absent (i.e. the circumventricular organs and choroid plexus) to trigger the production of second messengers (e.g. prostaglandins) to neural targets and induction of local cytokine production (Saper & Breder, 1994). In addition, *de novo* synthesis by resident cells within the CNS best explains the well-documented increase in brain levels of cytokines in response to peripheral infection or administration of immunogenic agents (including cytokines). This putative source also accounts for the presence of cytokine-producing cells (glial cells) and specific binding sites for the pro-inflammatory cytokines throughout the brain (Maier & Watkins, 1999; Vollmer-Conna, 2001). The exact mechanism for translation of the immune signal into a neural transmission is still unclear, but involves alterations in neuropeptide (e.g. corticotropin-releasing hormone, substance P, opioids) and neurotransmitter systems [noradrenaline, serotonin, gamma-aminobutyric acid (Rothwell & Hopkins, 1995; Konsman *et al.* 2002; Anisman & Merali, 2003)].

The elaboration of the concept of immunologically-induced sickness behaviour in recent years represents a major conceptual advance in our understanding of how sickness and recovery processes are organized in the brain. Sickness behaviour, similar to the 'fright–flight' response, is viewed as an adaptive survival strategy. However, in the same way as pathological fear and anxiety are debilitating, excessive or prolonged sickness behaviour may be deleterious. There has been much speculation on a possible role for cytokines in the pathogenesis of neuropsychiatric syndromes including depression and fatigue syndromes (Hickie & Lloyd, 1995; Vollmer-Conna *et al.* 1998; Maes, 1999; Charlton, 2000; Capuron *et al.* 2002; Anisman & Merali, 2003). This view is supported by a demonstration of the induction of debilitating, and at times treatment-limiting, sickness behaviour (including major depression and fatigue) concomitant with cytokine therapy in the treatment of cancer or hepatitis C (Renault & Hoofnagel, 1989; Capuron *et al.* 2002). However, such observations do not provide a useful model for the natural phenomenon of acute sickness behaviour as therapeutic use of cytokines typically employs very high concentrations of the proteins and the observed effects may be confounded by the underlying medical illness (Vollmer-Conna, 2001; Pollmacher *et al.* 2002).

To date, few studies have documented psychological and behavioural changes associated with acute infective illness in humans (e.g. Imboden *et al.* 1961; Smith *et al.* 1987; Westley-Wise *et al.* 1996; Vollmer-Conna *et al.* 1997; Capuron *et al.* 1999; Smith *et al.* 2000). Illness-associated changes in fatigue levels, pain perception, mood and cognitive abilities have been reported from this work, cytokine correlates, however, were not assessed and the infections were rarely documented serologically. Although significant advances in this field have been achieved through animal experimentation, there is a definite need for a better understanding of the phenomenon of sickness behaviour, and its immunological correlates, as it occurs naturally in sick humans. Without such a knowledge base, our ability to identify excessive, maladaptive manifestations of the phenomenon is limited and extrapolations to more chronic psychopathological conditions are premature.

The work presented here is derived from a substudy of an ongoing prospective cohort, the

Dubbo Infection Outcomes Study, which has been established in the region surrounding the rural township of Dubbo, in western New South Wales, Australia. The aim of the cohort study is to determine the pathogenesis of prolonged ill-health, including fatigue syndromes, following acute infection. The cohort is enrolling subjects with acute infection due to the mosquito-borne viral pathogen, Ross River virus (RRV) which causes a febrile illness marked by arthritis and rash; or Epstein–Barr virus (EBV), the causative agent of infectious mononucleosis or glandular fever; or the zoonotic infection Q fever caused by the rickettsia-like pathogen, *Coxiella burnetii*. Q fever is often a severe febrile illness associated with prominent headaches, constitutional symptoms and drenching sweats. This cohort offers a unique opportunity to characterize human sickness behaviour in the context of acute infection and to explore its biological basis. This paper reports results from two initial investigations. The objective of the first was to examine whether the pro-inflammatory cytokines (IL-1β and IL-6), measured in sera or cell culture supernatants, constitute useful and sensitive markers of the non-specific symptoms of sickness behaviour reported by patients recovering from acute Q fever. The aim of the second investigation was to replicate the initial findings in a larger group of subjects drawn from the remaining two cohorts (i.e. EBV or RRV infections).

METHOD

Subject recruitment and sample collection

Subjects were recruited in collaboration with general practitioners, the Centre for Population Health, as well as the public and private sector pathology services in the area. A system of prompt, but anonymous, coded notifications of EBV, RRV or Q fever IgM positive serological test results (indicating recent exposure) was established with each of the pathology laboratories in the Dubbo region. This allowed a research nurse to approach the general practitioners (with the coded information), and then, with permission, to approach their patients. All subjects were 16 years of age or over, and written informed consent was obtained prior to participation in the study. Subjects were excluded if they reported pre-existing immunological, or other, disorders likely to be associated with increased cytokine production, or if they were taking immunosuppressive medication. Blood sampling and data collection relevant to this study was performed by the research nurse at enrolment (as soon as possible after diagnosis – on average within 22 days of symptom onset). The blood samples were processed within 6 h of collection by separation and storage of serum and peripheral blood mononuclear cells (PBMC). The study was approved by the relevant institutional human research ethics committees.

Subjects

A total of 69 subjects from the three infective cohorts participated in this research. Of these, 21 (18 males, 3 females; mean age 40 years, range 16–60) suffered from acute Q fever. The male preponderance in this sample reflects the occurrence of Q fever in male meat workers. The remaining 48 subjects were included in the second, confirmatory analysis and consisted of 24 with EBV infection (13 males, 11 females; mean age 21 years, range 17–32) and 24 with RRV infection (9 males, 15 females; mean age 39 years, range 18–69).

Symptoms, behavioural and psychological measures

At enrolment, subjects completed self-report questionnaires to assess the dimensions of fatigue, physical symptoms and mood. These included the Somatic and Psychological Health Report (SPHERE; Hickie *et al.* 2001), and the General Health Questionnaire (GHQ-12; Goldberg & Williams, 1988). Questions were answered with a period prevalence of 'over the past two weeks'. In addition, the nurse completed a 17-item clinical checklist of infectious disease symptoms (referred to as Physical Symptom Checklist); with patients reporting the number of days each symptom was experienced. As the focus of this substudy was on acute sickness behaviour and its immunological correlates, a number of items/questions from these instruments were selected on the basis that they measure those non-specific symptoms reported in the literature as typical of sickness behaviour. The decisions regarding utilization of measures and the specific items to be included in analyses were made *a priori* and were guided by

availability of scores, as over the course of this long-term study somewhat different instruments were employed at different times. Given that no objective, validated measure of acute sickness behaviour exists, face validity to senior clinicians (I.H. and A.L.) also played a role in the selection of relevant items. Specifically, items chosen for analysis from the Physical Symptom Checklist related to the experience of fever ('had a fever'), malaise ('generally felt unwell'), anorexia ('lost your appetite'), excessive fatigue ('felt excessively tired' or 'exhausted'), myalgia ('had muscle aches and pains'), and arthralgia ('had pains in joints'); those chosen from the SPHERE related to headaches ('had a headache') and tired/heavy muscles ('muscles in arms and legs felt tired and heavy'); GHQ items were used for the more neuropsychiatric symptoms of anhedonia ('unable to enjoy day-to-day activities'), depression ('felt unhappy and depressed'), and impaired concentration ('unable to concentrate').

In addition, the Eysenck Personality Questionnaire (Eysenck & Eysenck, 1975) neuroticism scale was used to permit an assessment of a possible influence of this personality trait on the expression and reporting of symptoms.

Immunological measures

Blood collection, sample preparation and storage

Peripheral blood samples were collected and processed under strict endotoxin-minimized conditions to avoid artefactual stimulation of cytokine production *ex vivo*. Sera were frozen in multiple aliquots at -70 °C. PBMCs were separated by density gradient centrifugation, frozen in four or more aliquots of $5–10 \times 10^6$ cells and stored in liquid nitrogen for *in vitro* stimulation assays (see below). The cell viability after freeze–thaw in this protocol in our laboratory is consistently better than 85% when evaluated by Trypan blue dye exclusion. In addition, assays from fresh cells in comparison to frozen cells from the same donor have demonstrated highly concordant results in flow cytometric analyses of the leucocyte subpopulations and from analyses of *in vitro* cytokine production. For example, there was no significant difference ($p > 0{\cdot}5$, paired t test) in fresh *v.* frozen individual donor sample pairs, when assayed for lipopolysaccharide (LPS)-induced production of IL-6 (data not shown).

In vitro *stimulation assays*

After thawing, PBMCs were resuspended in Rosswell Park Memorial Institute medium (RPMI; Gibco, New York) supplemented with penicillin, streptomycin and L-glutamine (all from Trace Biosciences, Sydney, Australia), and low endotoxin, heat-inactivated fetal bovine serum (Trace Biosciences) at a concentration of 2×10^6 PBMCs/ml. The cell suspension was dispensed in quadruplicate into 96 well plates (Nunc, Denmark) in the presence or absence of LPS (10 ng/ml, from *Salmonella typhimurium*, Sigma). Cell cultures were held for 24 h at 37 °C before supernatants were harvested and stored at -70 °C.

Cytokine quantification

The levels of the pro-inflammatory cytokines IL-1β and IL-6 in sera and culture supernatants were quantified by sandwich ELISA in accordance with the manufacturer's instructions (Immunotech, Marseille, France).

Statistical analysis

The SPSS for Windows (Version 11) statistical package was used for descriptive and correlational analyses. Non-parametric Spearman rank order correlations were used to assess the relationship between symptoms and cytokine concentrations. The level of significance was set at $\alpha = 0{\cdot}05$.

RESULTS

Data in Fig. 1 show the percentage of patients in each of three cohorts reporting particular symptoms at enrolment into the study. It is apparent that at the point of first contact, the majority of subjects were still reporting symptoms typical of an acute infection. The symptom profiles vary somewhat across the three cohorts illustrating differences in the severity and pattern of illness expression of the three acute infections. Q fever was clearly the most severe, with fever, malaise, fatigue and neuropsychiatric symptoms universally reported. Across all three cohorts, the most frequently reported symptoms (100% of all patients) were anhedonia (inability to enjoy activities) and impaired concentration, with malaise and fatigue also

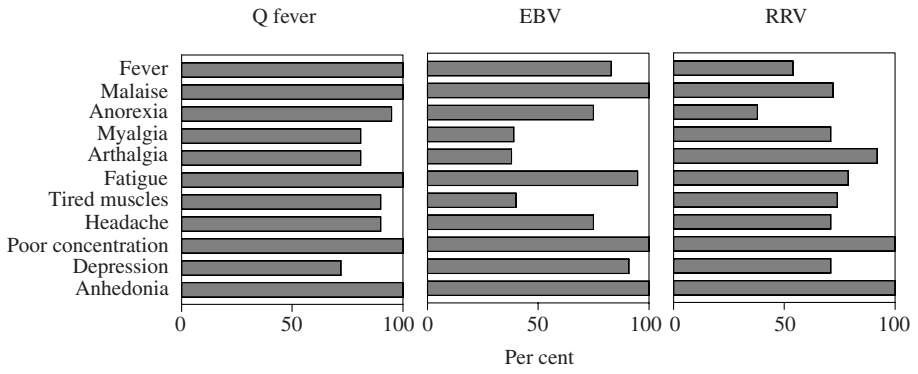

FIG. 1. Percentage of patients in the three cohorts reporting different symptoms of acute sickness.

being very commonly reported. There was little evidence that personality factors such as neuroticism consistently affected the expression of symptoms in our patients. Of the 11 symptoms assessed in this study, only depressed mood ($r_S = 0{\cdot}31$, $p = 0{\cdot}02$) and inability to concentrate ($r_S = 0{\cdot}32$, $p = 0{\cdot}01$) correlated significantly with neuroticism scores. No significant associations were found between neuroticism scores and concentrations of either IL-1β or IL-6.

Study 1 (Q fever)

Associations between symptoms of acute sickness and cytokine concentrations in serum, as well as unstimulated, and LPS-stimulated, cell culture media are shown in Table 1. Notably, strong, positive correlations with many self-reported sickness symptoms were obtained with the levels of IL-1β and IL-6 in unstimulated cell cultures indicative of 'spontaneously' released cytokines. Interestingly, comparable correlations were not found with serum values. In the unstimulated supernatants, higher levels of IL-1β were associated with the physical symptoms [fever ($r_S = 0{\cdot}44$, $p = 0{\cdot}05$), malaise ($r_S = 0{\cdot}53$, $p = 0{\cdot}02$) arthralgia and myalgia (both $r_S = 0{\cdot}56$, $p = 0{\cdot}01$)], whereas the levels of IL-6 to correlated more strongly with the neuropsychiatric symptoms of depression ($r_S = 0{\cdot}51$, $p = 0{\cdot}02$) and poor concentration ($r_S = 0{\cdot}54$, $p = 0{\cdot}02$), and also with headache ($r_S = 0{\cdot}57$, $p = 0{\cdot}01$). Levels of both cytokines correlated significantly with items relating to fatigue [excessive fatigue: IL-1β ($r_S = 0{\cdot}47$, $p = 0{\cdot}04$), IL-6 ($r_S = 0{\cdot}45$, $p = 0{\cdot}04$); tired muscles: IL-1β ($r_S = 0{\cdot}52$, $p = 0{\cdot}02$), IL-6 ($r_S = 0{\cdot}60$, $p = 0{\cdot}007$), and anhedonia: IL-1β ($r_S = 0{\cdot}62$, $p = 0{\cdot}004$), IL-6 ($r_S = 0{\cdot}48$, $p = 0{\cdot}03$)]. No significant correlation was found between cytokine levels and the symptom of anorexia. As shown in Table 1, correlational analysis of symptoms and cytokine levels in sera and stimulated culture media did not produce any additional information of significance.

Table 1. *Correlations (Spearman's r values) between symptoms reported by patients with acute Q fever ($n = 21$) and cytokine (IL-1β and IL-6) concentrations in sera, and supernatants from unstimulated (medium), or LPS-stimulated (LPS) PBMC cultures. Significant values are in bold*

Reported symptoms	IL-1β			IL-6		
	Serum	Medium	LPS	Serum	Medium	LPS
Fever	0·30	**0·44**	**0·45**	0·30	0·20	0·03
Malaise	0·22	**0·53**	0·26	0·04	0·43	0·13
Anorexia	0·35	0·38	0·38	0·20	0·18	0·06
Arthralgia	0·21	**0·56**	0·16	0·02	0·42	0·41
Myalgia	0·08	**0·56**	0·29	0·01	**0·47**	0·25
Excessive fatigue	0·18	**0·47**	0·06	−0·07	**0·45**	0·25
Tired/heavy muscles	0·14	**0·52**	0·25	−0·13	**0·60**	0·10
Headache	0·09	0·38	0·42	0·03	**0·57**	0·07
Poor concentration	−0·14	0·32	0·33	0·07	**0·54**	0·29
Depression	0·00	0·38	0·16	0·02	**0·51**	0·31
Anhedonia	0·30	**0·62**	0·13	0·25	**0·48**	−0·01

LPS, lipopolysaccharide; PMBC, peripheral blood mononuclear cell.

Study 2 (EBV/RRV)

Data obtained from patients in the remaining two cohorts were analysed together. In addition

Table 2. *Correlations between non-specific symptoms of acute illness and cytokine (IL-1β and IL-6) concentrations in unstimulated cell culture supernatants in 48 patients with EBV or RRV infection. These are shown alongside comparable data from the Q fever sample (presented earlier). Significant values are in bold*

	EBV and RRV ($n=48$)		QF ($n=21$)	
Symptoms	IL-1β	IL-6	IL-1β	IL-6
Fever	0·28	**0·35**	**0·44**	0·20
Malaise	0·17	0·18	**0·53**	0·43
Anorexia	0·01	0·04	0·38	0·18
Arthralgia	**0·29**	0·24	**0·56**	0·42
Myalgia	**0·32**	0·27	**0·56**	**0·47**
Excessive fatigue	**0·39**	**0·42**	**0·47**	**0·45**
Tired/heavy muscles	**0·41**	**0·36**	**0·52**	**0·60**
Headache	0·27	**0·33**	0·38	**0·57**
Poor concentration	0·21	**0·37**	0·32	**0·54**
Depression	**0·35**	**0·44**	0·38	**0·51**
Anhedonia	**0·47**	**0·41**	**0·62**	**0·48**

EBV, Epstein–Barr virus; RRV, Ross River virus; QF, Q fever.

to producing a larger sample size, this approach was considered justified on theoretical grounds. The non-specific symptoms, collectively termed sickness behaviour are, by definition, not specific to a particular infection, but rather common to all acute illnesses.

The pattern of results obtained from correlational analysis of the EBV/RRV data was similar to that obtained from the Q fever sample. As before, the most significant results were produced from correlations involving cytokine concentrations in culture supernatants of unstimulated PBMCs. Therefore, Table 2 shows results pertaining to unstimulated culture supernatants only. To illustrate the similarity in findings, the corresponding Q fever results are presented alongside these findings from the subjects with EBV or RRV infections. Similar to the Q fever sample, IL-1β was associated with arthralgia ($r_S=0{\cdot}29$, $p=0{\cdot}05$), and myalgia ($r_S=0{\cdot}32$, $p=0{\cdot}02$), and IL-6 correlated with poor concentration ($r_S=0{\cdot}37$, $p=0{\cdot}01$) and headaches ($r_S=0{\cdot}33$, $p=0{\cdot}02$). In this sample, reported fever was more strongly related to IL-6 ($r_S=0{\cdot}35$, $p=0{\cdot}02$), whereas the correlation with IL-1β was close to significance ($r_S=0{\cdot}28$, $p=0{\cdot}055$). Both cytokines correlated reliably with items relating to fatigue [excessive fatigue: IL-1β ($r_S=0{\cdot}39$, $p=0{\cdot}01$), IL-6 ($r_S=0{\cdot}42$, $p=0{\cdot}005$); tired muscles: IL-1β ($r_S=0{\cdot}41$, $p=0{\cdot}005$), IL-6 ($r_S=0{\cdot}36$, $p=0{\cdot}01$)], anhedonia: IL-1β ($r_S=0{\cdot}47$, $p=0{\cdot}001$), IL-6 ($r_S=0{\cdot}40$, $p=0{\cdot}007$), and also depression IL-1β ($r_S=0{\cdot}35$, $p=0{\cdot}02$) and IL-6 ($r_S=0{\cdot}44$, $p=0{\cdot}003$). Again, no reliable association between reported anorexia and concentrations of either cytokine was found.

DISCUSSION

These results document that the pro-inflammatory cytokines, IL-1β and IL-6, constitute sensitive markers of CNS-mediated, non-specific symptoms associated with serologically confirmed Q fever, EBV and RRV infections. This is an important finding, as the literature is devoid of evidence documenting a link between levels of peripheral cytokines, produced naturally as part of the host response to infection, and the experience of sickness behaviour in humans. Moreover, the correlational data suggest a quantitative relationship between the strength of the peripheral molecular signals of sickness and the extent to which sickness behaviour manifests in the individual.

As expected symptoms characteristic of sickness behaviour were a common experience reported by subjects in all three cohorts. This finding is consistent with the universal nature of sickness behaviour initially described in animals (Hart, 1988). Although different pathogenic microorganisms may cause acute infections of varying severity and somewhat different symptom profiles, the core behavioural and psychological manifestations of sickness have been described as stable across infection type and species. Interestingly, the symptoms most consistently reported across the cohorts (100% of all subjects) were neuropsychiatric in nature and included anhedonia and impaired concentration. This confirms and extends previous reports suggesting that mood changes and impaired cognitive performance are an integral part of human sickness behaviour (Vollmer-Conna *et al.* 1997; Capuron *et al.* 1999; Smith *et al.* 2000). As many patients continue with their daily work routine throughout an illness such as glandular fever, or Q fever, a better understanding of the full extent of cognitive deficits associated with acute infections is needed as this may well have implications for

road and work place safety. We found little evidence to support a consistent influence of stable personality characteristics, such as trait emotionality, or neuroticism, on the subjective experience of symptoms. Although depressed mood and poor concentration did correlate with neuroticism scores this is not surprising as one would expect a measure of emotionality to relate to symptoms that reflect, after all, an emotional state (i.e. depression). Alternatively it is possible that, the experience of neuropsychiatric symptoms, synonymous with a depressed mood state, when subjects were acutely ill influenced the manner in which subjects answered the questions on the trait measure. Eysenck (1994) himself noted that scores in neuroticism must always be interpreted in the light of the motivational context at the time of collection. Overall this finding supports the notion that acute sickness behaviour is essentially a biological phenomenon whose function is adaptive.

The results obtained from the Q fever sample suggest a degree of specificity in the associations between cytokines and symptoms. Elevated concentrations of IL-1β appeared to relate more strongly to physical symptoms of sickness (e.g. fever, malaise, aches and pains), and IL-6 to neuropsychiatric symptoms (e.g. poor concentration, depression). To a lesser degree this tendency was also apparent in the results obtained from patients with EBV or RRV infections. Although intuitively appealing, these findings should be interpreted with some caution. To begin with, the levels of IL-1β and IL-6 in unstimulated cultures were highly correlated (Q fever: $r_S = 0{\cdot}84$; EBV/RRV: $r_S = 0{\cdot}92$) which makes it difficult to draw definite conclusions regarding any specific role of particular cytokines in the induction of sickness symptoms. Given the pleiotropism and redundancy of the cytokine network, it is unlikely that precise contributions of individual cytokines to sickness behaviour can easily be determined. There is some evidence suggesting that TNF-α and IL-1β, synthesized very early in the immune response, are more potent inducers of the behavioural correlates than those released later (i.e. IL-6), and act in synergy to produce sickness behaviour (Dantzer *et al.* 1996). The role of IL-6 in sickness behaviour is likely to be complex, as recent evidence suggests that IL-6 has both pro-and anti-inflammatory functions (Papanicolaou *et al.* 1998). Its anti-inflammatory actions include the induction of natural antagonists to IL-1 and TNF-α, and activation of the HPA axis. IL-6 is also secreted in response to distress and disrupted sleep (Papanicolaou *et al.* 1998; Lutgendorf *et al.* 1999; Irwin, 2002). It is possible that correlations between IL-6 and particular symptoms, rather than reflecting specificity, may signal the presence of these symptoms somewhat later in the recovery process when levels of IL-6 are higher. More definitive answers to questions relating to the possible specificity of cytokines and their temporal relations to the emergence and resolution of aspects of sickness behaviour require more systematic, detailed and longitudinal analysis of this phenomenon in sick humans.

In the present study, the most robust correlations were obtained with data relating to cytokine levels in unstimulated PBMC cultures. This pattern has been previously demonstrated in a study comparing cytokine production in patients with acute Q fever to that of healthy control subjects (Capo *et al.* 1996). Significant between-group differences were documented for all cytokines, but only when spontaneous production in unstimulated cell cultures was assessed. These differences were not evident in LPS-stimulated cultures, potentially reflecting the potent capacity of LPS to induce cytokines *in vitro* thus obscuring subtle differences in illness-associated cytokine release. The finding that measurement of circulating cytokines in serum may not correlate well with symptoms has previously been documented, and is proposed to be due to the fact that cytokines have short half-lives and predominantly act locally at the site of infection (Pollmacher *et al.* 2002). Nevertheless, it appears that PBMCs allowed to release cytokines during overnight incubation *ex vivo* may provide a sensitive instrument for further exploration of the study of acute sickness behaviour.

The main limitation of the present study is that the sickness behaviour data derived from responses to the SPHERE, GHQ and the Physical Symptom Checklist at enrolment into the study related to a period prevalence covering the prior 2 weeks of acute illness, whereas the cytokine measurements are likely to reflected

proteins produced *in vivo* and cleared from the body with half-lives measured in hours. These data therefore do not provide an optimal index of symptom prevalence at the actual time of blood sampling, but rather constitute a marker of severity of recent illness experience. Furthermore, the symptom data were obtained by self-report, rather than by direct observations such as measurement of fever, real time records of pain levels and documented intake of analgesic medications. Despite these caveats, the positive results obtained from correlational analyses suggest that during an illness episode with ongoing cytokine production, these symptom parameters are relatively stably present.

Another limitation is that levels of TNF-α were not assessed in this study. As sample availability was constrained, we chose to concentrate on IL-1β, which has been consistently associated with sickness behaviour in animals (Hart, 1988; Dantzer *et al.* 1996) and IL-6, because of its different temporal release pattern and possible association with neuropsychiatric symptoms (Papanicolaou *et al.* 1998; Lutgendorf *et al.* 1999; Irwin, 2002). The potential relationship between TNF-α and sickness behaviour will be the subject of future studies.

In summary, the results indicate that peripheral pro-inflammatory cytokines, produced naturally as part of the host response to infection represent sensitive markers of the experience of sickness behaviour in humans. This relationship was found to be consistent across infections caused by different micro-organisms. Additional research is needed to characterize more fully the spectrum of sickness behaviour in humans and to elucidate its immunological correlates. Such investigations should also include more detailed recording of the altered behaviours associated with acute illness (such as reduced food intake, altered sleep patterns and social withdrawal) as well as formal assessment of cognitive impairment. Longitudinal analyses of the natural course of sickness behaviour, studying the emergence, maintenance and resolution of different aspects of the phenomenon, would greatly advance our understanding of putative pathological variants, such as post-viral syndromes and major depression.

The elaboration of the concept of sickness behaviour in recent years has already enhanced our appreciation of the physiological and psychological impact of diseases like chronic infections and inflammatory conditions, as well as cancer and their treatments. In future, this work may lead to the development of management practices that complement the innate disease-coping strategies and may be particularly beneficial when dealing with viruses or drug resistant bacteria (Hart, 1988). Furthermore, it may provide a novel, but more complete perspective of the pathophysiological complexities underlying poorly understood neuropsychiatric syndromes.

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DECLARATION OF INTEREST

None.

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