Postinfective Fatigue Syndrome Is Not Associated with Altered Cytokine Production

Uté Vollmer-Conna,[1] Barbara Cameron,[2] Dusan Hadzi-Pavlovic,[1] Kristi Singletary,[4] Tracey Davenport,[3] Suzanne Vernon,[4] William C. Reeves,[4] Ian Hickie,[3] Denis Wakefield,[2] and Andrew R. Lloyd,[2] for the Dubbo Infective Outcomes Study Group[a]

[1]School of Psychiatry and [2]Centre for Infection and Inflammation Research, School of Medical Sciences, University of New South Wales, and [3]Brain & Mind Research Institute, Sydney University, Sydney, Australia; and [4]Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

Peripheral blood specimens and clinical data were obtained over a 12-month period from subjects in the Dubbo Infection Outcomes Study to examine cytokine production in postinfective fatigue syndrome. Ex vivo production of 8 cytokines was examined in 22 case patients and in 42 control subjects who recovered promptly. No significant differences were found. Ongoing production of the cytokines examined does not play a role in postinfective fatigue syndrome.

Despite intensive research efforts, the pathophysiology of the enigmatic clinical disorder chronic fatigue syndrome (CFS) remains obscure, and effective therapies are not available [1]. Prospective cohort studies have empirically verified the existence of a postinfective fatigue syndrome (PIFS) consistent with CFS that is triggered by infection with Epstein-Barr virus (EBV), Ross River virus (RRV), or *Coxiella burnetii* (the causative agent of Q fever) [2–5].

Hypotheses that abnormalities in the immune response underpin the pathogenesis of CFS have been extensively explored. However, a systematic review suggested that reported alterations in T cell responses, cytokine levels, and natural killer cell activity were inconsistent and rarely correlated with the clinical condition [6]. Substantial heterogeneity among patients fulfilling the diagnostic criteria for CFS [7, 8] is the likely reason for such inconsistent findings. Accordingly, we established a disease model for the onset and evolution of CFS, by means of a prospective cohort study of subjects observed from the onset of documented acute EBV infection, RRV infection, or Q fever: the Dubbo Infection Outcomes Study (DIOS) [5]. In this study, PIFS was documented in 27% of subjects at 3 months, with 11% fulfilling diagnostic criteria for CFS at 6 months after detailed medical and psychiatric evaluation, to exclude alternative causes for illness. The PIFS illness was stereotyped across infections, suggesting that the host response, rather than microbiological factors, determined ongoing symptoms.

In subjects from DIOS studied early in the febrile phase of illness, the severity of symptoms, including fatigue, correlated with levels of the proinflammatory cytokines IL-1β and IL-6, in unstimulated PBMC cultures, indicative of cytokines "spontaneously" released ex vivo [9]. Thus, the present study aimed to analyze cytokine production in patients with PIFS and matched control subjects who had recovered uneventfully from the same infection, to resolve whether ongoing, aberrant cytokine production mediates prolonged fatigue states.

Subjects and methods. Participants were enrolled after presentation with acute febrile illness and detection of IgM antibodies against EBV, RRV, or *C. burnetii.* These provisional serologic diagnoses were confirmed by testing acute- and convalescent-phase serum samples [5].

Subjects were assessed at 1, 2, 3, 6, and 12 months after the onset of infection. At each visit, physical and psychological health was assessed, and a blood sample was collected. The 34-item Somatic and Psychological Health Report [10] was used to monitor symptoms. A score of ≥ 3 (maximum score, 12) on the empirically derived subscale SOMA was used to record PIFS, because this reliably predicts disability and reflects patients' and doctors' reports of reasons for presentation to primary care [10]. Subjects were classified as having provisional PIFS if their SOMA scores at all time points up to and including 3 months exceeded this threshold. When symptoms persisted beyond 6 months, structured medical and psychiatric assessments and laboratory investigations were undertaken to exclude alternative explanations for ongoing illness and to designate CFS diagnosis (termed "confirmed PIFS") [1]. The items "days in bed" and the number of "days out of role" (Brief Disability Questionnaire) served to index functional impairment [11]. The factor score coefficients (symptom weights) from the original study [5] were applied to each subject's responses on the Somatic and Psychological Health Report to quantify illness

Received 3 April 2007; accepted 15 May 2007; electronically published 6 August 2007.

Study group members are listed at the end of the text.

Reprints or correspondence: Dr. Ute Vollmer-Conna, Dept. of Human Behaviour/Psychiatry, University of New South Wales, UNSW Sydney 2052, New South Wales, Australia (ute@unsw.edu.au).

Clinical Infectious Diseases 2007; 45:732–5

1058-4838/2007/4506-0009$15.00
DOI: 10.1086/520990

severity in 6 domains: acute sickness, irritability, fatigue, musculoskeletal pain, neurocognitive disturbance, and mood disturbance.

The participants were 22 subjects with confirmed PIFS (11 patients with EBV infection, 6 with RRV infection, and 5 with Q fever) and 42 control subjects (17 patients with EBV infection, 14 with RRV infection, and 11 with Q fever) matched for age from the same cohort who had recovered within 6 weeks of symptom onset. Written informed consent was obtained from all subjects. The relevant institutional review boards approved the study.

Blood samples were collected and processed under strict endotoxin-minimized conditions. PBMCs were separated (Lymphoprep; AXIS-SHIELD) and cryopreserved in RPMI (Trace Biosciences) with 10% dimethylsulfoxide (Sigma) and 50% autologous plasma, and aliquots were stored in liquid nitrogen. Serum was separated and stored in aliquots at −80°C.

After being thawed, PBMCs were resuspended in RPMI (GIBCO) supplemented with 10% heat-inactivated fetal bovine serum (Trace Biosciences) at 2×10^6 PBMCs/mL. The cell suspension was dispensed into 96-well plates (Nunc) in medium only (unstimulated cultures), lipopolysaccharide (10 ng/mL; *Salmonella typhimurium;* Sigma), or mouse anti-human, anti-CD3, and anti-CD28 Dynabeads added at a ratio of 4 beads to 1 cell ratio (Dynal). Plates were held for 24 h at 37°C. Supernatants were stored at −80°C until being subjected to the multiplex immunoassay. Concentrations of the cytokines IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, TNF-α, and IFN-γ in serum and culture supernatants were analyzed (BioPlex; BioRad).

The linear mixed-effects models procedure (in SPSS for Windows, version 14; SPSS Software) was chosen to analyze the influence of PIFS status on longitudinal patterns of symptom severity and cytokine production. Because the PIFS illness was stereotyped across different infective triggers, acute-phase cytokine production was comparable across the 3 infections, and neither psychological nor microbial factors were predictive of PIFS [5, 9], the data set was collapsed across infections for analysis. Student's t test was used for group comparisons of age, duration, and disability, and the χ^2 test was used to assess differences in the sex and case/control distributions.

Results. Subjects with PIFS did not differ from the control subjects with regard to age (mean ± SD, 32 ± 14 vs. 32 ± 15 years; $P = .99$) or sex ($P = .34$ for the ratio of male to female subjects). Of the 64 participants, 28 were infected with EBV, 20 were infected with RRV, and 16 had Q fever. The proportion of PIFS cases to controls (0.66 to 0.34) did not differ between infections ($P = .76$). The first assessment in this study (denoted as 1 month) occurred a mean (±SD) of 29 ± 8 days after onset of symptoms. This interval did not differ between case patients and control subjects ($P = .46$). The mean duration of the entire illness (±SD) was 40 ± 19 weeks for case patients and 5 ± 3 weeks for control subjects ($P < .001$). The mean number of days in bed (±SD) for patients with PIFS was 18 ± 15, compared with 7 ± 8 days among control subjects ($P = .03$). Subjects with PIFS also reported significantly more days out of role (PIFS group, 44 ± 31 days; control subjects, 16 ± 13 days; $P = .01$).

As expected, the analysis of symptom factor scores (figure 1*A*) established that PIFS status was associated with more-severe symptoms in all 6 domains ($P > .001$ for each). A significant main effect for time was evident for acute sickness ($P = .03$), fatigue ($P = .001$), and musculoskeletal pain ($P = .006$), indicating a decrease in symptom severity over time in these domains. Older age was associated with higher levels of musculoskeletal pain ($P = .003$) and neurocognitive disturbance ($P = .02$).

Serum cytokine levels were almost exclusively less than the detection limit of the assay system (8–15 pg/mL) and did not differ between the groups (data not shown); therefore, the analysis focused on culture supernatants. Mixed-effects modeling did not reveal any between-group differences in cytokine levels over time. These results were consistent across all culture conditions ($P > .05$ for all) (figure 1*B*). Older age was associated with higher levels of IL-4 ($P = .02$) in anti-CD3/anti-CD28–stimulated cultures and with higher levels of IL-1β ($P = .003$), IL-6 ($P = .02$), and TNF-α ($P = .02$) in lipopolysaccharide-stimulated cultures.

Discussion. This comprehensive analysis of 8 different cytokine responses in PIFS failed to reveal any association with disease status, thus providing strong evidence against the hypothesis that prolonged fatigue is associated with altered cytokine production. These results are concordant with our recent finding that specific antiviral immune responses in the EBV subcohort of the DIOS did not reveal substantive differences between subjects with PIFS and those who recovered promptly [12]. It remains possible that testing with other cytokine-inducing stimuli and/or assessing a broader panel may implicate other cytokines in PIFS.

Irrespective of PIFS status, older subjects reported more pain and neurocognitive symptoms and showed an increased pro-inflammatory response to lipopolysaccharide challenge, as well as a bias toward Th2-type lymphokine production (IL-4) in anti-CD3/anti-CD28–stimulated cultures. Similar responses have previously been attributed to immune senescence [13].

Consistent with earlier reports from DIOS, the present data indicate that symptoms of PIFS originate in the acute phase of the infections and persist over weeks to months [5, 12]. Acute infections are typically accompanied by a cluster of stereotyped symptoms, including fever, fatigue, hypersomnia, hyperalgesia, mood disturbance, and impaired concentration [9]. With the exception of fever, it is these symptoms that typify the PIFS illness. Accumulated animal data indicate that, in acute infec-

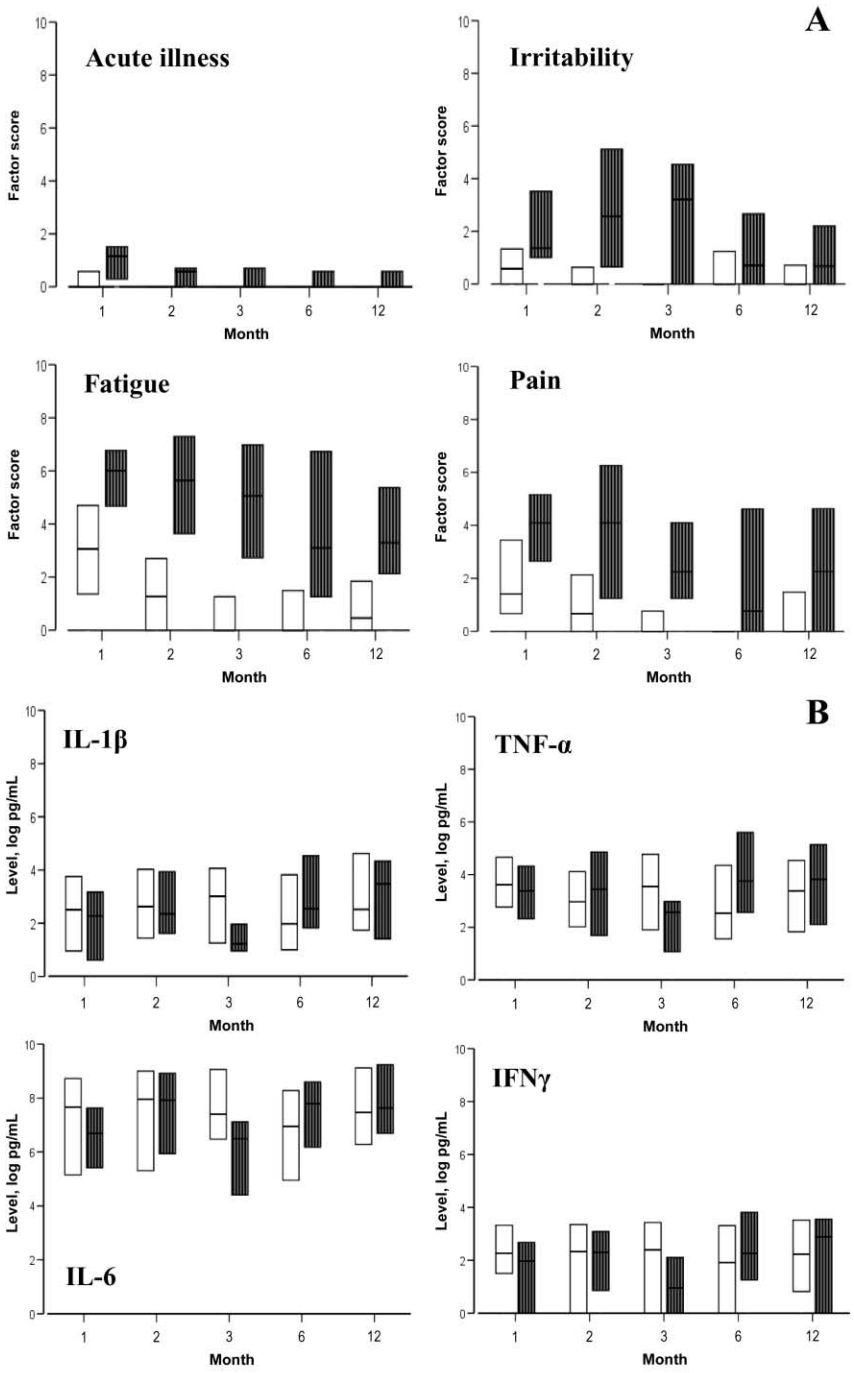

Figure 1. *A,* Severity of illness in different symptom domains over a 12-month period for subjects with postinfective fatigue syndrome (PIFS; *shaded columns*) and control subjects who recovered uneventfully from the same infections *(open columns). B,* Cytokine levels produced in unstimulated peripheral blood mononuclear cell cultures collected over a 12-month period from subjects with PIFS *(shaded columns)* and control subjects *(open columns). Bars,* median; *box extremities,* 25th and 75th percentiles.

tion, these symptoms are mediated by the action of proinflammatory cytokines on the brain [14]. We have confirmed this finding in subjects from DIOS studied early in the febrile phase [9]. Nevertheless, no such association was found with comparable symptoms in the prolonged course of PIFS.

Although the present study cannot exclude a role for other untested factors or for cytokines at very low levels or produced in specific microenvironments [15, the evidence suggests that immunologic stressors such as acute infections constitute a "trigger" rather than the "driver" of symptoms in PIFS. Con-

sequently, alternative hypotheses of pathogenesis need to be explored, including persistent production of inflammatory mediators by activated glial cells in the absence of ongoing peripheral stimuli or sensitization of neural response pathways to physiological signals from the periphery [15].

Study group members. The additional investigators of the Dubbo Infection Outcomes Study Group include (in alphabetical order): Heather Dunckley (Australian Red Cross Blood Service, Sydney), Andrew Geczy (Australian Red Cross Blood Service, Sydney), Ray Harris (University of South Australia, Adelaide), Rajiv Khanna (Queensland Institute for Medical Research, Brisbane), Barrie Marmion (Institute for Medical and Veterinary Science, Adelaide), and Bill Rawlinson (Prince of Wales Hospital, Sydney).

Acknowledgments

We gratefully acknowledge the support of the general practitioners, the public health unit, and the diagnostic pathology services in the Dubbo region and the enduring cooperation of the participants in the research. We also acknowledge the assistance provided by Dr. Rosane Nisenbaum in the statistical analysis.

Financial support. National Health and Medical Research Council of Australia Project Grants (157092 and 157062); Meat & Livestock, Australia; and Centers for Disease Control and Prevention (Cooperative Research Agreement U50/CCU019851-01).

Potential conflicts of interests. All authors: no conflicts.

References

1. Fukuda K, Straus SE, Hickie I, et al. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Ann Intern Med **1994**; 121: 953–9.
2. White PD, Thomas JM, Amess J, et al. Incidence, risk and prognosis of acute and chronic fatigue syndromes and psychiatric disorders after glandular fever. Br J Psychiatry **1998**; 173:475–81.
3. Buchwald DS, Rea T, Katon W, et al. Acute infectious mononucleosis: characteristics of patients who report failure to recover. Am J Med **2000**; 109:531–7.
4. Candy B, Chalder T, Cleare AJ, et al. Predictors of fatigue following the onset of infectious mononucleosis. Psychol Med **2003**; 33:847–55.
5. Hickie I, Davenport TA, Wakefield D, et al. Viral and non-viral pathogens precipitate post-infective and chronic fatigue syndromes: a prospective cohort study. BMJ **2006**; 333:575–8.
6. Lyall M, Peakman M, Wessely S. A systematic review and critical evaluation of the immunology of chronic fatigue syndrome. J Psychosom Res **2003**; 55:79–90.
7. Wilson A, Hickie I, Hadzi-Pavlovic D, et al. What is chronic fatigue syndrome? Heterogeneity within an international multicentre study. Aust N Z J Psychiatry **2001**; 35:520–7.
8. Vollmer-Conna U, Aslakson E, White PD. An empirical delineation of the heterogeneity in chronic unexplained fatigue in women. Pharmacogenomics **2006**; 7:355–64.
9. Vollmer-Conna U, Fazou C, Cameron B, et al. Production of pro-inflammatory cytokines correlates with the symptoms of acute sickness behaviour in humans. Psychol Med **2004**; 34:1289–97.
10. Hickie IB, Davenport TA, Hadzi-Pavlovic D, et al. Development of a simple screening tool for common mental disorders in general practice. Med J Aust **2001**; 175:S10–7.
11. von Korff M, Ustun T, Ormel J, Kaplan I, Simon G. Self-report disability in an international primary care study of psychological illness. J Clin Epidemiol **1996**; 49:297–303.
12. Cameron B, Bharadwaj M, Burrows J, et al. Prolonged illness following infectious mononucleosis is associated with altered immunity but not elevated viral load. J Infect Dis **2006**; 193:664–71.
13. Weng NP. Aging of the immune system: how much can the adaptive immune system adapt? Immunity **2006**; 24:495–9.
14. Hart BL. Biological basis of the behavior of sick animals. Neurosci Biobehav Rev **1988**; 12:123–37.
15. Pollmacher T, Haack M, Schuld A, et al. Low levels of circulating inflammatory cytokines—do they affect human brain functions? Brain Behav Immun **2002**; 16:525–32.